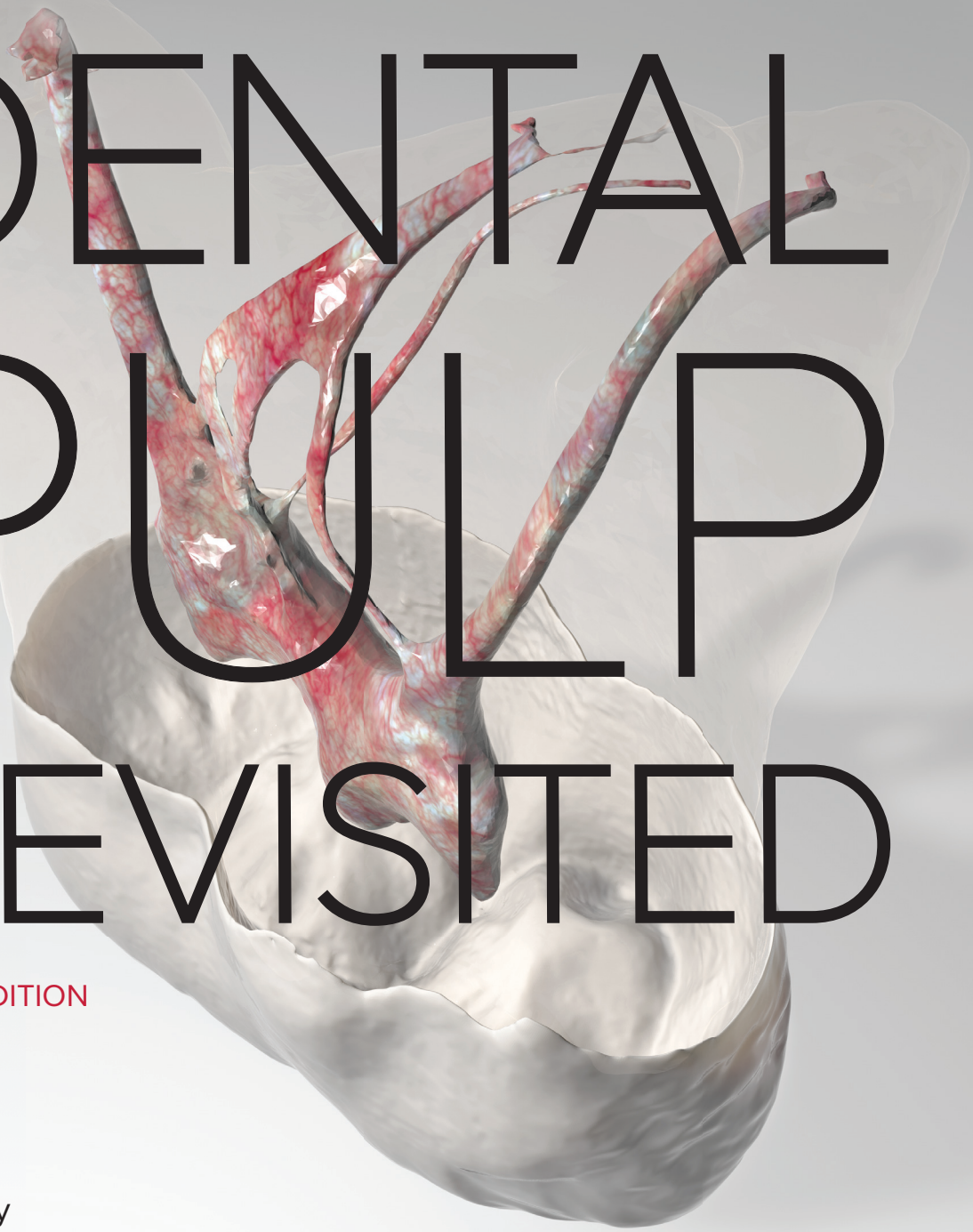
Seltzer and Bender's

# DENTAL PULP REVISITED



THIRD EDITION

Edited by
**Scott B. McClanahan, DDS, MS**
**Ronald Ordinola Zapata, DDS, MS, PhD**

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Seltzer and Bender's Dental Pulp Revisited
*Third Edition*

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**Library of Congress Cataloging-in-Publication Data**
Names: McClanahan, Scott B., editor. | Ordinola Zapata, Ronald, editor.
Title: Seltzer and Bender's dental pulp revisited / edited by Scott B. McClanahan and Ronald Ordinola Zapata.
Other titles: Dental pulp
Description: Third edition. | Batavia, IL : Quintessence Publishing Co., Inc., [2025] | Includes bibliographical references and index. | Summary: "Reviews the most current scientific information regarding the interactions that take place within the pulp space and the effects of those interactions on clinical endodontic practice"-- Provided by publisher.
Identifiers: LCCN 2024046243 | ISBN 9781647241889 (hardcover)
Subjects: MESH: Dental Pulp | Endodontics--methods | Dental Pulp Diseases | Dental Pulp--physiology
Classification: LCC RK351 | NLM WU 230 | DDC 617.6/342--dc23/eng/20241115
LC record available at https://lccn.loc.gov/2024046243

A CIP record for this book is available from the British Library.
ISBN: 978-1-64724-188-9

QUINTESSENCE PUBLISHING USA

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Quintessence Publishing Co, Inc
411 N Raddant Road
Batavia, IL 60510
www.quintessence-publishing.com

5 4 3 2 1

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Editor: Leah Huffman
Production: Angelina Schmelter
Design: Sue Zubek

Printed in Korea

*Cover illustration courtesy of Marco A. Versiani, DDS, MSc, PhD.*

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*Edited by*


**Scott B. McClanahan, DDS, MS**
Professor Emeritus
Division of Endodontics
University of Minnesota School of Dentistry
Minneapolis, Minnesota

**Ronald Ordinola Zapata, DDS, MS, PhD**
Associate Professor
Director, Graduate Endodontics
Division of Endodontics
University of Minnesota School of Dentistry
Minneapolis, Minnesota



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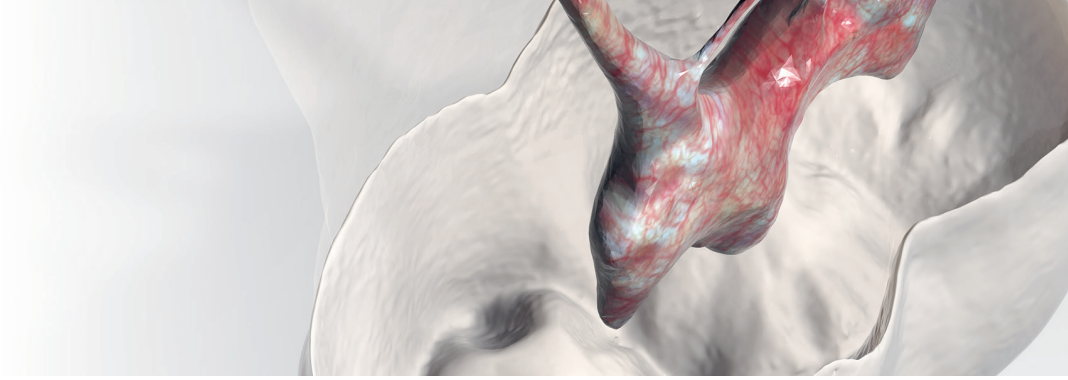

# Foreword

*"A title and a tribute for the ages"*

It has been 58 years since the original dental pulp text was published under the title *The Dental Pulp: Biologic Considerations in Dental Procedures*. Drs Sam Seltzer and I. B. Bender were the authors of this landmark text. For many years this textbook served as the singular authoritative source for all things dealing with the pathology of the dental pulp and periradicular tissues. However, as time passed, expanded research, new discoveries, innovation in materials and equipment, and a vastly expanded dental community wrought significant changes to the in-depth knowledge of endodontic disease along with its diagnosis and treatment. Knowing this, we can legitimately ask why we continue to honor and recognize Drs Seltzer and Bender in the title of the third edition of this textbook when their contributions occurred so long ago. That question can be easily answered by evaluating the enormous impact these two extraordinary individuals had on both our understanding of endodontic disease and our efforts to effectively diagnose and treat this widespread affliction. They effectively changed the course and nature of inquiry and our understanding of pulpal and periradicular disease forever.

Both Sam Seltzer and I. B. Bender were strong-willed, bright, outgoing, and highly productive professionals who had an indefatigable urge to find out the *why* for what they observed. Over many years, they disagreed so fervently on some issues that for extended periods they could not work together. However, each time there was a schism between the two, some invisible force pulled them back together to ask some of the most important questions for the specialty, to develop some of the most important methods to answer those questions, and to present information that destroyed long-held beliefs and empirical practices.

This writer has vivid memories of my first meeting in 1969 when I was introduced to Drs Seltzer and Bender by my endodontic fellowship mentor, Dr Malcomb Davis, a board-certified Navy endodontist, at the annual session of the American Association of Endodontists in Chicago. Sam and I. B. would hold court in a hotel suite for a long evening of debate and discussion with other endodontic luminaries of the time. Loud and heated debate on many topics would ensue among the 30 to 35 endodontic educators, researchers, and clinicians in the room. Topics ranged from the value of culturing to the optimal length of root canal fillings to why common practices in endodontic treatment did not produce optimal or even expected outcomes. By the end of the evening, the Seltzer and Bender arguments and positions rose to the top of the intellectually stimulating fray.

In a moving and beautifully written memorial tribute for Drs Bender and Seltzer published in the *International Endodontic Journal* in 2004, Gunnar Bergenholtz wrote that these two men became synonymous with critical and challenging observations on the nature, diagnosis, and management of the disease processes associated with the dental pulp. Together, Seltzer and Bender contributed more than 40 papers, and even more independently, that delved critically into pulpal and periradicular disease and the often futile or flawed efforts the dental research and clinical communities made to accurately diagnose, understand, and successfully treat these disease entities. A large majority of their papers provided new, creative, and sometimes iconoclastic methods for examining, testing, and interpreting difficult, seemingly unanswerable and troublingly normal and pathologic occurrences. They redirected and promoted research efforts worldwide to employ new investigative methodologies and to freshly examine and interpret that which was inexplicable.

In 1957, the well-known American social psychologist Leon Festinger first described the theory of cognitive dissonance. His description of cognitive dissonance was what occurs when people's thoughts and feelings are inconsistent with their behavior, resulting in an uncomfortable and disharmonious feeling. He went on to say that because the experience of dissonance is so uncomfortable, people are highly motivated to try to reduce their dissonance and that that effort was a fundamental need.

Within a few short years after the initial description of cognitive dissonance by Dr Festinger, Drs Seltzer and Bender, acutely aware of the theory, recognized that the endodontic basis for the commonly accepted treatment concepts in endodontics was essentially flawed, or worse yet, founded on no basis at all. Yet mainstream thinking and practice continued to embrace opinion or empirical justifications for the clinical practice of endodontics. This



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muddled and disharmonious state fit the Festinger description of cognitive dissonance like a glove. It provided the impetus for Seltzer and Bender to describe these incongruities and then challenge the long-held concepts in a paper aptly titled "Cognitive Dissonance." What ensued was a major shift in emphasis in endodontics away from empiricism to research and practices rooted in outcomes based on the scientific method. Especially noteworthy, it ultimately spelled the end of root canal culturing as a shibboleth of endodontic treatment.

Clinicians, academics, and researchers will never agree on which Seltzer and Bender paper contributed most to the clear understanding of pulpal and periradicular disease and the actions that need to be taken to eliminate these closely related pathologic entities. It serves a better purpose to identify a few of the areas of investigation that together propelled pulp and periradicular biology and pathology to new levels of understanding and treatment direction. Some of those areas included the connected relationship between pulp and periodontal disease, the radiographic detection of osseous lesions, the validity of correlating clinically collected diagnostic information and the histologic or pathohistologic status of the pulp, and the biologic reactions of the periapical tissues to endodontic procedures. Obviously this is an abbreviated list. However, it highlights some of the areas that challenged the worldwide dental community to change its empirical thinking to sound scientific practices that guided the diagnosis and treatment of pulpal and periapical disease for generations to come.

The fact that we continue to honor Sam Seltzer and I. B. Bender in the title of this third edition of *Seltzer and Bender's Dental Pulp Revisited* is not only appropriate but compelling. The extraordinary impact of these two giants on the advancement of endodontics and dental pulp biology is immeasurable.

**M. Lamar Hicks, DDS, MS**

Former Chair and Program Director
I.B. Bender Division of Endodontics
The Albert Einstein Medical Center
Philadelphia, Pennsylvania

Former Dean
Naval Postgraduate Dental School
Bethesda, Maryland

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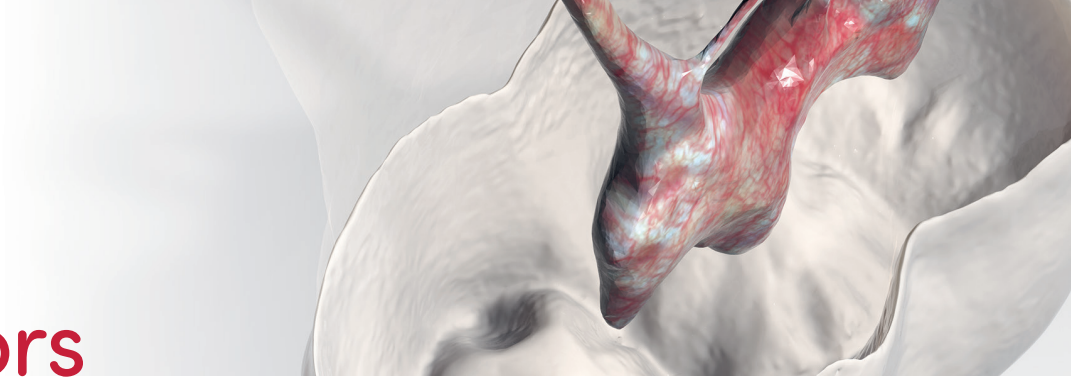

# Contributors


**Anita Aminoshariae, DDS, MS**
Professor
Department of Endodontics
Case Western Reserve University School of Dental Medicine
Cleveland, Ohio

**Maria Teresa Arias-Moliz, BDS, MSc, PhD**
Associate Professor
Facultad de Odontología
University of Granada
Granada, Spain

**Javier Caviedes-Bucheli, DDS, MSc**
Professor and Researcher
Pontificia Universidad Javeriana School of Dentistry
Bogota, Colombia

**Luis Eduardo Chavez de Paz, DDS, MS, PhD**
Private practice
Stockholm, Sweden

**Paul R. Cooper, PhD**
Dean and Professor of Oral Biology
Department of Oral Sciences
Sir John Walsh Research Institute
Faculty of Dentistry
University of Otago
Dunedin, New Zealand

**Iryna H. Daline, DMD, MSD, MS**
Private practice
Austin, Texas

**Anibal Diogenes, DDS, MS, PhD**
Professor and Chair
Department of Endodontics
School of Dentistry
UT Health San Antonio
San Antonio, Texas

**Katherine Divine, DDS, MS**
Private practice
Rochester, Minnesota

Assistant Clinical Professor
Division of Endodontics
University of Minnesota School of Dentistry
Minneapolis, Minnesota

**Scott L. Doyle, DDS, MS**
Private practice
Woodbury, Minnesota

Associate Clinical Professor
Division of Endodontics
University of Minnesota School of Dentistry
Minneapolis, Minnesota

**Henry F. Duncan, DDS, PhD**
Professor
Division of Restorative Dentistry and Periodontology
Dublin Dental University Hospital
Trinity College Dublin
Dublin, Ireland

**Ashraf F. Fouad, DDS, MS**
Professor and Chair, Department of Endodontics
Director, Advanced Educational Program in Endodontics
School of Dentistry
The University of Alabama at Birmingham
Birmingham, Alabama

**Francisco Wanderley Garcia de Paula-Silva, DDS, MS, PhD**
Associate Professor
Ribeirão Preto School of Dentistry
University of São Paulo
São Paulo, Brazil

**Franklin García-Godoy, DDS, MS, MS, PhD, PhD**
Professor
Department of Bioscience Research
College of Dentistry
University of Tennessee Health Science Center
Memphis, Tennessee

**Gustavo Pompermaier Garlet, DDS, MS, PhD**
Professor
Department of Biological Sciences
School of Dentistry of Bauru
University of São Paulo (FOB/USP)
Bauru, São Paulo, Brazil

**Brenda P.F.A. Gomes, DDS, MS, PhD**
Professor
Division of Endodontics
Piracicaba Dental School
Universidade Estadual de Campinas
Piracicaba, São Paulo, Brazil

**Lisha Gu, DDS, PhD**
Professor
Department of Operative Dentistry and Endodontics
Guanghua School of Stomatology
Sun Yat-Sen University
Guangzhou, China

**James L. Gutmann, DDS**
Professor Emeritus
Department of Restorative Sciences and Endodontics
Texas A&M University College of Dentistry
Dallas, Texas

**Sivakami Rethnam Haug, BDS, PhD**
Associate Professor
Chair, Section for Endodontics
Department of Clinical Dentistry
University of Bergen Medical School
Bergen, Norway

**James D. Johnson, DDS, MS**
Professor and Chair
Department of Endodontics
University of Washington School of Dentistry
Seattle, Washington

**Bekir Karabucak, DMD, MS**
Professor, Chair, and Postdoctoral Program Director
Department of Endodontics
University of Pennsylvania School of Dental Medicine
Philadelphia, Pennsylvania

**Asma A. Khan, BDS, PhD**
Professor
Department of Endodontics
School of Dentistry
UT Health San Antonio
San Antonio, Texas




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**Syngcuk Kim, DDS, MPhil, PhD, MD (Hon)**
Professor and Associate Dean of Global Affairs
Department of Endodontics
University of Pennsylvania School of Dental Medicine
Philadelphia, Pennsylvania

**Alan S. Law, DDS, PhD**
Private practice
Woodbury, Minnesota

Research Professor
Division of Endodontics
University of Minnesota School of Dentistry
Minneapolis, Minnesota

**Ariadne Letra, DDS, MS, PhD**
Professor
Department of Oral and Craniofacial Sciences
Center for Craniofacial Genetics
University of Pittsburgh School of Dental Medicine
Pittsburgh, Pennsylvania

**Linda Levin, DDS, PhD**
Private practice
Durham, North Carolina

**Jorge N.R. Martins, DDS, MSc, PhD**
Faculdade de Medicina Dentária
Universidade de Lisboa
Lisbon, Portugal

**Scott B. McClanahan, DDS, MS**
Professor Emeritus
Division of Endodontics
University of Minnesota School of Dentistry
Minneapolis, Minnesota

**Hugo Roberto Munoz, DDS, MSc**
Professor and Researcher
Department of Endodontics
University of San Carlos
Guatemala City, Guatemala

**Peter E. Murray, BSC (Hons), PhD**
Professor
College of Dental Medicine
Nova Southeastern University
Fort Lauderdale, Florida

**W. Craig Noblett, DDS, MS**
Clinical Associate Professor
Director, Division of Endodontics
University of Minnesota School of Dentistry
Minneapolis, Minnesota

**David H. Pashley, DMD, PhD**
Professor Emeritus
The Dental College of Georgia
Augusta University
Augusta, Georgia

**Jorge Perdigão, DMD, MS, PhD**
Professor
Division of Operative Dentistry
University of Minnesota School of Dentistry
Minneapolis, Minnesota

**Renato Menezes Silva, DMD, MS, PhD**
Professor and Chair
Department of Endodontics
University of Pittsburgh School of Dental Medicine
Pittsburgh, Pennsylvania

**Carlos Alberto de Souza Costa, DMD, MS, PhD**
Professor
Department of Physiology and Pathology
UNESP/Araraquara School of Dentistry
Araraquara, Brazil

**Franklin Tay, BDSc (Hons), PhD**
Professor and Chair
Department of Endodontics
The Dental College of Georgia
Augusta University
Augusta, Georgia

**Marco A. Versiani, DDS, MSc, PhD**
Dental Specialty Center
Brazilian Military Police
Belo Horizonte, Minas Gerais, Brazil

**Dustin Weitz, DMD, MS**
Private practice
Moscow, Idaho and Clarkston, Washington

**Ronald Ordinola Zapata, DDS, MS, PhD**
Associate Professor
Director, Graduate Endodontics
Division of Endodontics
University of Minnesota School of Dentistry
Minneapolis, Minnesota

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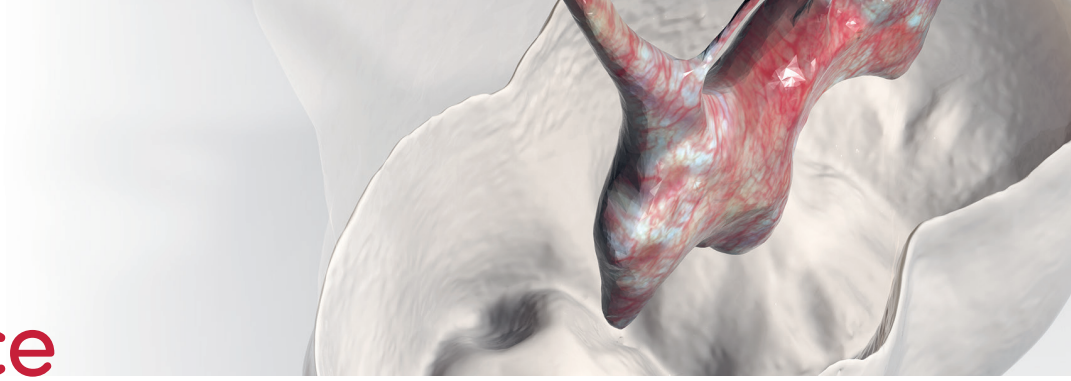

# Preface

As discussions with Quintessence about a third edition of *Seltzer and Bender's Dental Pulp* moved forward, we thought carefully about the immense significance of a new edition and our tremendous responsibility as editors. Once the decision was made and we had the green light to proceed, we outlined our goals and shared them with the chapter authors as the chapter titles and chapter order were configured. The goals were as follows:

1. The new edition should be readable.
2. It should be focused on clinical correlations.
3. The chapters should include the most current scientific information available at the time of submission to the editors.
4. The book should be targeted primarily for use in graduate endodontic education.

We wanted the third edition to be the must-read book for all endodontic graduate students. The chapter authors were amazing and excelled in their efforts to accomplish these goals. For their efforts, we are eternally grateful. We hope you enjoy the fruits of their labors.

**Scott B. McClanahan**
**Ronald Ordinola Zapata**

## A Note on Citations

To maintain the citation standards of endodontic literature while ensuring readability, all sources are cited parenthetically in the text, but years are only included to differentiate between multiple sources for a given author/group in that chapter's reference list. For example, "Ordinola-Zapata et al" indicates the only study by that group in the reference list, whereas "Ordinola-Zapata et al 2024" indicates the 2024 source by this group as opposed to the others in the reference list for that chapter. Multiple sources are separated by commas.



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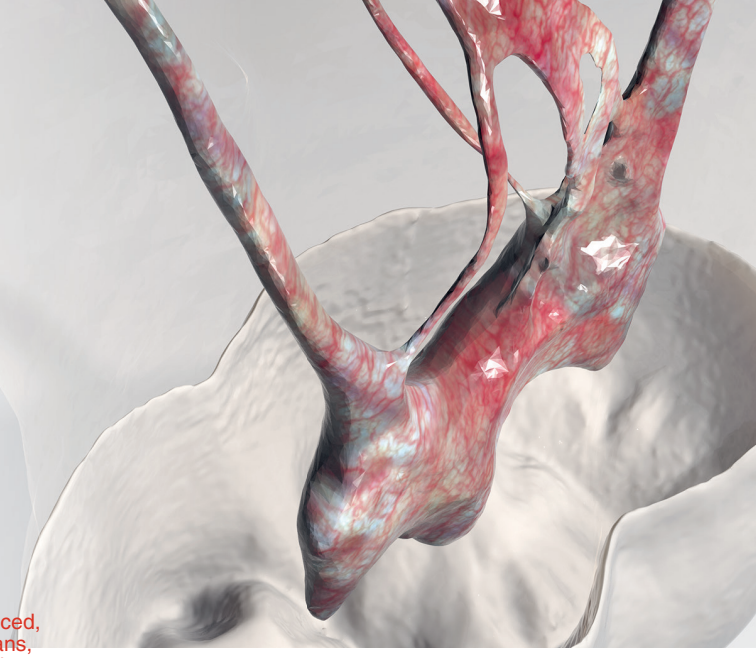
# 1

# THE DENTIN-PULP COMPLEX

Lisha Gu
David H. Pashley
Franklin Tay



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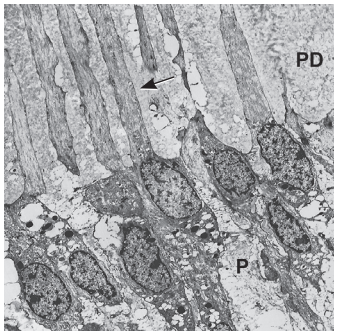
The dental pulp and dentin are often referred to as a functional unit, the *dentin-pulp complex* (formerly *pulpodentin complex*). This chapter describes the structure of dentin and summarizes its chemical and physical properties. Some of the mechanical properties of dentin that account for the development of dentinal cracks are also highlighted to help readers appreciate why fractures frequently occur in coronal and radicular dentin. The chapter concludes with a description of the inward diffusion of irrigants and the outward flow of dentinal fluid that are the hallmarks of dentin permeability.

## Structure of the Dentin-Pulp Complex

The dentin and the pulp are functionally integrated as one tissue. When dentin is exposed following a breach in the integrity of the overlying enamel or cementum, the natural compartmentalization of the dentin and pulp is lost, and the two tissues functionally become a fluid-filled continuum. Bacterial substances and noxious materials then diffuse across the dentin through this fluid medium to produce pulpal reactions (Pissiotis and Spångberg, Bergenholtz, Galler et al). The pulp responds to these chemical stimuli in the short term by mounting an acute inflammatory response, which produces an outward movement of both fluid (Andrew and Matthews, Vongsavan and Matthews 2007) and macromolecules (Maita et al, Pashley and Pashley 1991). In the long term, pulpal tissues produce tertiary dentin as a biologic response to reduce the permeability of the dentin-pulp complex and restore it to its original sequestration (Byers and Yoon Lin, Bjørndal et al). Radioactive tracer experiments have been used to demonstrate the continuity of dentinal fluid–pulpal fluid circulation in exposed dentin in vivo (Pashley 1979), which illustrates both the importance of pulpal blood flow in clearing pulpal interstitial fluids of exogenous material and the behavior of the dentin-pulp complex functioning as an integrated unit (Pashley 1996).

Odontoblasts are terminally differentiated cells that form the tubular dentin matrix. Their cell bodies reside in the pulp chamber (Fig 1-1), but their processes extend various distances through unmineralized, freshly secreted predentin into the mineralized matrix (Goldberg et al). Although odontoblasts are generally perceived as formative cells that secrete the different components of dentin, they are also immune cells associated with innate immunity, as well as sensor cells for cold (Kawashima and Okiji, Bernal et al). Odontoblasts behave as immune cells because of the pattern-recognition receptors—such as toll-like receptors, nucleotide-binding oligomerization domain (NOD) receptors, and NOD-like receptor protein 3 (NLRP3)—found on their surfaces (Yumoto et al). External cold and heat stimuli activate thermosensitive ion channels from the transient receptor potential (TRP) superfamily that are present on nociceptive nerve endings and odontoblasts to initiate pain responses (Solé-Magdalena et al; Fig 1-2). Other mechanosensitive




◀ **FIG 1-1** Transmission electron micrograph of the human odontoblast layer at the junction of the pulp (P) and predentin (PD). The odontoblasts are organized as a layer of palisade cells. Each cell sends an odontoblast process *(arrow)* through the predentin toward the periphery.

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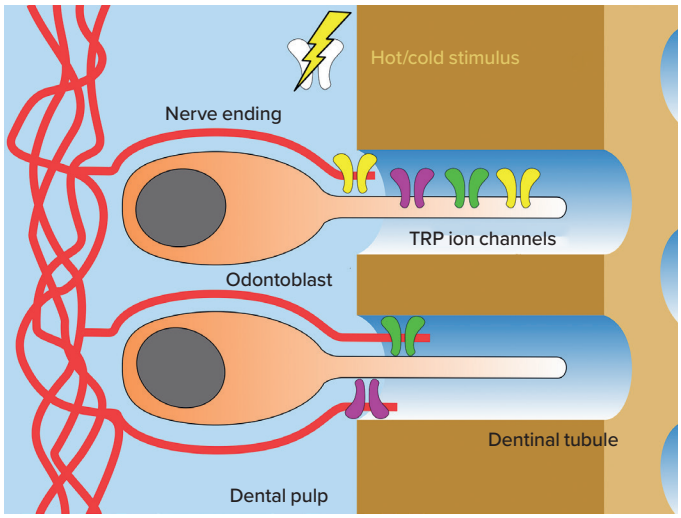
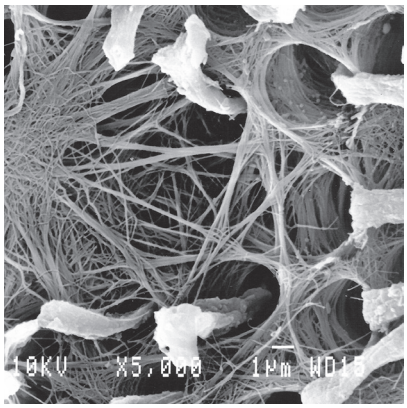
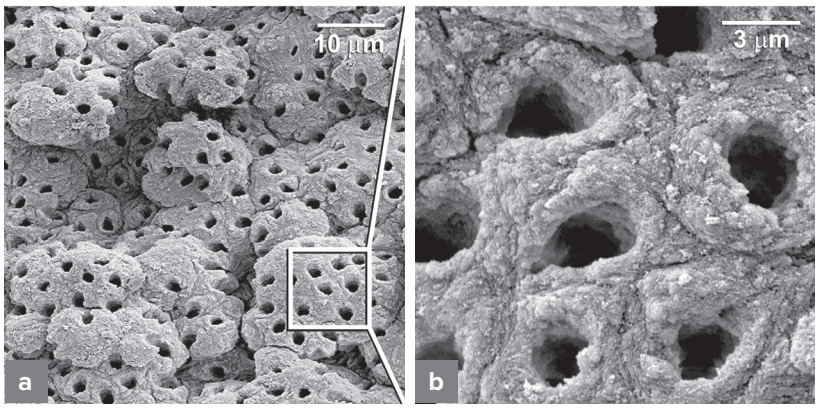



◀ **FIG 1-2** Thermosensitive ion channels on the surface of dental primary afferent sensory nerve endings and odontoblasts are activated by external heat or cold stimuli. These ion channels are derived from the TRP superfamily of cation channels and may include members of different subfamilies, such as TRPV (vanilloid), TRPM (melastatin), TRPA (ankyrin), and TRPC (canonical). Activation of these ion channels in the odontoblast results in the outflow of adenosine triphosphate (ATP) and possibly other mediators from the cell. The extracellular mediators bind to specific receptors in dental pulp neurons. Excitation of these neurons triggers the transmission of nociceptive signals to the trigeminal ganglion and subsequently to the cerebral cortex, where they are perceived as pain.



▲ **FIG 1-3** Predentin is a network of unmineralized collagen fibrils that are approximately 100 nm in diameter. These fibrils are arranged perpendicular to the odontoblast processes.




▲ **FIG 1-4** *(a)* The mineralization front can be seen after the predentin is dissolved with sodium hypochlorite. This mineralization front is organized into hemispheric units known as *calcospherites*. *(b)* High magnification of the box in *a*. Each calcospherite contains many dentinal tubules that are 2 to 3 µm in diameter.

piezo ion channels and acid-sensing ion channels (ASICs) that are present on the nerve endings and odontoblasts are responsible for dental nociception caused by the rapid movement of dentinal fluid or the deformation of microstructure (Lee et al, Sun et al).

If the odontoblast layer is removed, the odontoblast processes are also sometimes removed from the unmineralized tubules of predentin (Fig 1-3). Removal of the predentin with 5% sodium hypochlorite allows visualization of the underlying mineralization front that exhibits characteristic hemispheric structures known as *calcospherites* (Mishima and Kozawa). Mineralized dentinal tubules that are almost 3 µm in diameter can be seen within these calcospherites (Fig 1-4). Calcospherites undergo fusion as the body ages (Arora et al).

# Dentin

## Dentin morphology

Dentin is a porous biologic composite composed of apatite filler particles in a collagen matrix (Fig 1-5). This mineralized matrix is produced by odontoblasts, which begin secreting collagen at the dentinoenamel junction (DEJ) and then grow centripetally while trailing odontoblast processes. Acid etching or chelation with ethylenediaminetetraacetic acid (EDTA) removes the highly mineralized peritubular dentin, thereby enlarging the tubule orifice and removing the apatite crystallites from around the collagen fibrils. This results in exposure of the type 1 collagen–rich intertubular dentin matrix (Fig 1-6).

There are three types of dentin: primary dentin, secondary dentin, and tertiary dentin. Primary dentin is the original tubular dentin that is formed prior to the eruption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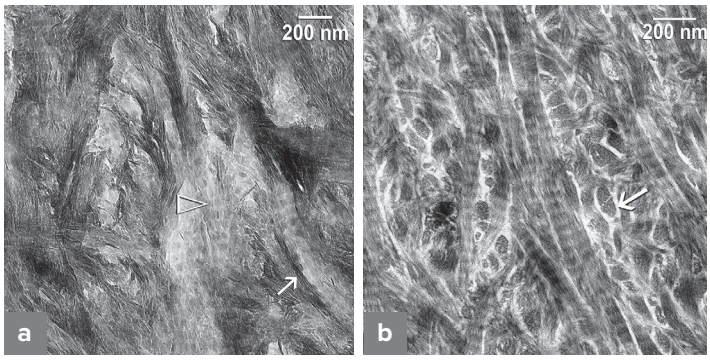
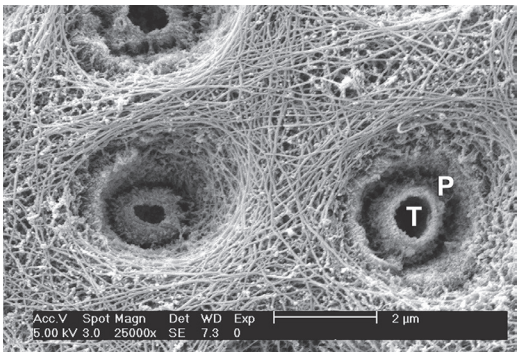



▲ **FIG 1-5** Transmission electron micrographs. *(a)* Unstained, mineralized dentin showing the deposition of apatite crystallites within the collagen fibrils (intrafibrillar mineralization; *open arrowhead*) and between the collagen fibrils (interfibrillar mineralization; *arrow*). *(b)* Stained, demineralized dentin showing the same striations or banding characteristics as the stained collagen fibrils. Dissolution of the interfibrillar apatite minerals results in empty interfibrillar spaces *(arrow)*.

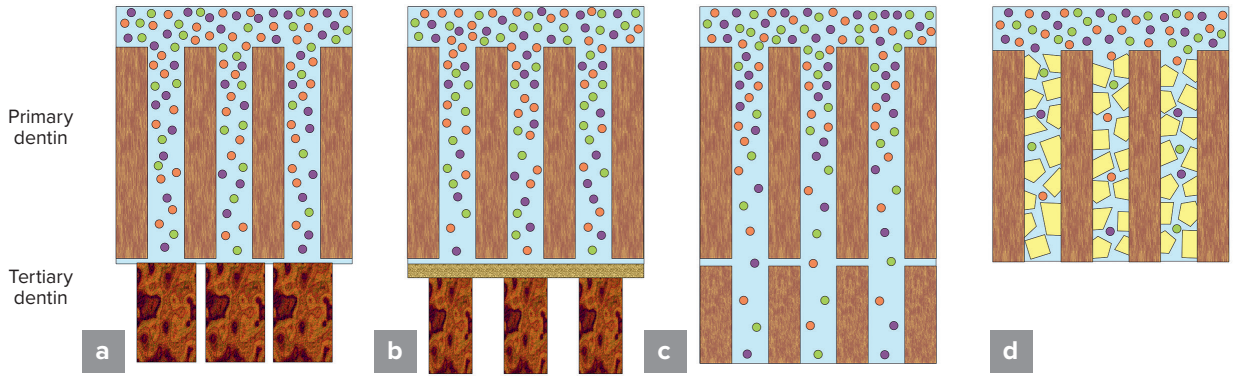



▲ **FIG 1-6** Acid etching dentin removes the highly mineralized peritubular dentin (P) and strips apatite crystallites off the intertubular collagen fibrils, permitting the true fibrillar nature of the intertubular dentin matrix to be seen. T = dentinal tubule.

of a tooth. Mantle dentin is the outer layer of primary dentin. It is slightly (about 4%) less mineralized but is similar in composition to primary dentin found in aged teeth (Senawongse et al 2006). Mantle dentin is about 150 µm wide and comprises the first dentin laid down by newly differentiated odontoblasts. These cells may not be completely differentiated, or they may have relatively short odontoblast processes, resulting in slightly less than ideal mineralization (Tjäderhane et al).

Secondary dentin is the same circumpulpal dentin as primary dentin except that it is formed after the completion of root formation. The major difference between primary and secondary dentin is that secondary dentin is secreted more slowly. Because the same odontoblasts form both types of dentin, the tubules are continuous. Over decades, a large amount of secondary dentin is formed on the roof and floor of the pulp chamber, causing the chamber to become shallower. Similarly, the formation of secondary dentin causes the dimensions of the root canal to become increasingly smaller with age. The presence of cellular processes on odontoblasts makes primary and secondary dentin tubular in nature.

The third type of dentin is tertiary dentin. It is also known as *irritation dentin*, *irregular secondary dentin*, *reactionary dentin*, and *reparative dentin* (Smith et al, Neves and Sharpe, Tziafas, Tziafas et al; Fig 1-7). Tertiary




▲ **FIG 1-7** The tubular nature of various types of dentin. Primary dentin is shown above the less tubular tertiary dentin. The small dots indicate the concentration of potentially noxious substances diffusing across the dentin. *(a)* The original odontoblasts have been destroyed, and the newly differentiated odontoblasts in the reparative dentin do not line up with the original primary dentin, which greatly reduces its permeability. *(b)* Sometimes the newly formed odontoblasts lack a process and form a layer of tubular dentin that can reduce the permeability of the dentin to near zero. *(c)* Other times injured odontoblasts do not die but simply make more dentin at a faster rate, thereby increasing dentin thickness and reducing its permeability. *(d)* Intraluminal crystalline deposits within the dentinal tubules may reduce dentin permeability.

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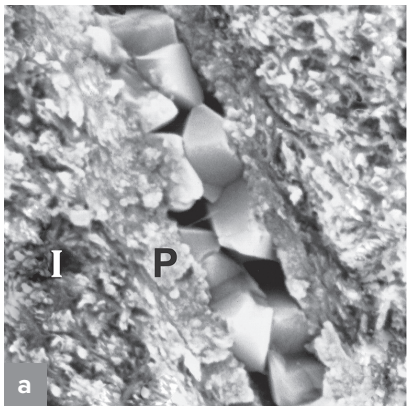
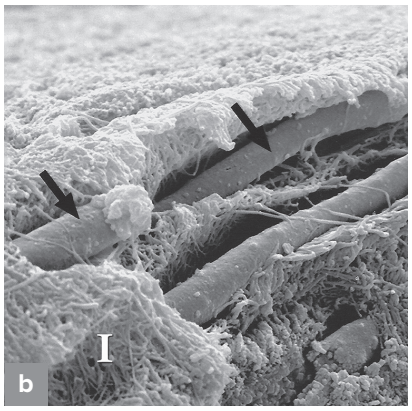
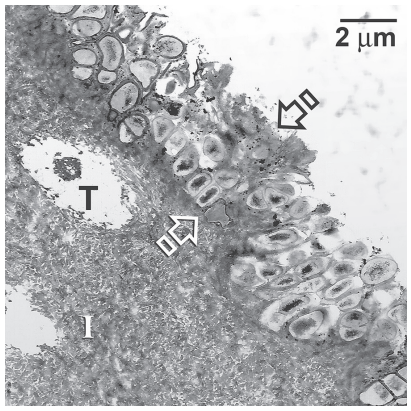



▲ **FIG 1-8** Examples of intraluminal crystalline deposits that contribute to reducing dentin permeability. *(a)* Large whitlockite crystals that are composed of magnesium-substituted β-tricalcium phosphate. *(b)* Cores of fine crystalline deposits *(arrows)* that resemble peritubular dentin (P) completely occlude the dentinal tubules. They are resistant to acid etching and are separated from the adjacent demineralized, acid-etched intertubular dentin (I) due to dehydration shrinkage. The presence of intraluminal deposits makes sclerotic dentin almost impermeable.




▲ **FIG 1-9** Although bacteria can invade dentinal tubules, they are seldom found in the tubules (T) at the extreme front of carious attacks, probably because of the outward movement of dentinal fluid, which contains immunoglobulins. The *open arrows* indicate the layer of bacteria biofilm. I = intertubular dentin.

dentin is formed when a tooth is subjected to trauma or irritation (eg, exposed hypersensitive cervical dentin, carious dentin, traumatic cavity preparation).

Odontoblasts are highly polarized cells (Chang et al). The nucleus of a secretory odontoblast is oriented toward the pulpal end of the cell, and numerous organelles are present in its supranuclear part (Couve). Because the circumference of the most peripheral part of the crown or root of a tooth is much larger than that of the pulp chamber or root canal space, odontoblasts are forced closer together as they continue to lay down dentin, developing a pseudostratified columnar layer in parts of the coronal pulp, especially over the pulp horns (Arana-Chavez and Massa). In radicular dentin, odontoblasts are cuboidal in the root canal and become flat near the apex (Marion et al). The convergence of dentinal tubules at the pulp lends a unique structural organization to dentin that has profound functional consequences.

Each dentinal tubule is an inverted cone that is smallest at the DEJ and largest at the pulp. Although each tubule starts with a diameter of nearly 3 µm, a collagen-free and hypermineralized cuff of peritubular dentin within each tubule (see Fig 1-6) narrows the lumen of the tubule to as little as 0.6 to 0.8 µm in superficial dentin (Garberoglio and Brännström). The large amount of peritubular dentin found in superficial dentin near the DEJ is due in part to the fact that it is "older" than middle or deep dentin. Thus, the width of peritubular dentin decreases as tubules are followed inward toward the pulp. Very close to the pulp, where there is no peritubular dentin, the tubule (luminal) diameter is almost 3 µm (Mjör 1985). Within the root canal, the mean diameter of dentinal tubules decreases from 4.32 µm in the coronal part to 3.74 µm in the middle part and 1.73 µm in the apical part of the canal space (Lo Giudice et al).

The composition of peritubular dentin differs from intertubular dentin in the following ways:

- Peritubular dentin lacks collagen fibrils.
- Peritubular dentin contains an organic scaffold, composed predominantly of glycosaminoglycans (Bertassoni et al 2012b), that is embedded with apatite crystallites. The carbonated apatite crystallites have a higher crystallinity and are almost five times harder than intertubular dentin (Kinney et al 1996).

Little is known about the biologic control of peritubular dentin apposition. Although it is a very slow process, apposition can be accelerated by occlusal abrasion and other forms of pulpal irritation, such as caries (Tjäderhane et al).

Although the complete composition of dentinal fluid is unknown, presumably it contains an ion product of calcium and phosphate that is near or above the solubility product constants for different forms of calcium phosphate (Larsson et al, Lundgren et al). This fluid tends to create different forms of mineral deposits within the dentinal tubules (Fig 1-8). Whereas the flow rate of dentinal fluid has no effect on dentin demineralization, the composition of dentinal fluid is an important determinant of the rate of mineral dissolution during lesion formation and dentinal caries progression (Özok et al).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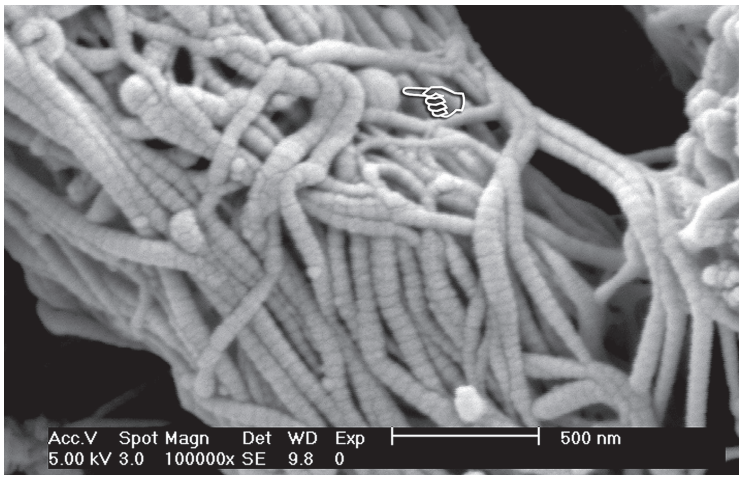
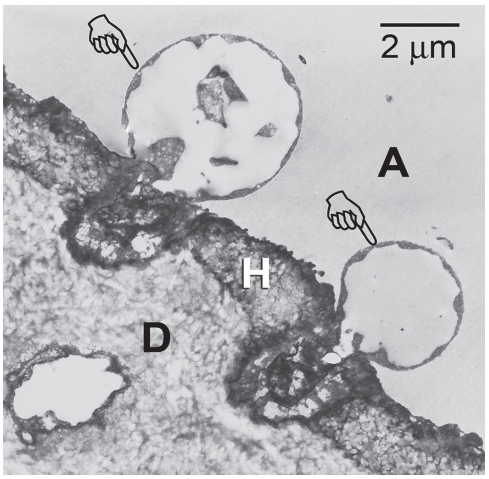



▲ **FIG 1-10** Large bundles of intraluminal collagen help to reduce the functional diameter of dentinal tubules and hence the permeability of dentin. These collagen bundles also trap any suspended bacteria *(pointer)* as fluid flows through the tubules.




▲ **FIG 1-11** Transmission electron micrograph depicting the transudation of dentinal fluid and tubular contents *(pointers)* from the tubules during dentin bonding to acid-etched vital dentin. The fluid droplets are trapped within the dentin adhesive (A) and act as stress raisers that adversely affect the integrity of resin-dentin bonds. H = hybrid layer; D = intertubular dentin.

The permeability properties of dentinal tubules indicate that they have much smaller functional dimensions than their actual microscopic dimensions (Michelich et al 1978). Although the microscopic diameter of dentinal tubules at the DEJ is reported to be between 0.5 and 0.9 µm, they function as though they are 0.1 µm in diameter. Thus, dentin can prevent passage of 99.8% of a bacterial suspension of streptococci that are approximately 0.5 µm in diameter when pressure is applied to the solution (Michelich et al 1980). This phenomenon explains why there are no bacteria in the tubules at the extreme front of carious attacks. Although bacteria can invade dentinal tubules (Love and Jenkinson), they do not invade as fast or as far in vital dentin (Ricucci et al; Fig 1-9). This is due to out-flowing dentinal fluid that contains plasma proteins and immunoglobulins (Knutsson et al, Hahn and Overton). Immunoglobin G1 (IgG1) is the predominant immunoglobulin subclass found in uninfected dentinal tubules beneath shallow and deep caries (Hahn and Best). Although fluid shifts occur across dentin, the fluid is virtually sterile because of intratubular deposits of mineral and collagen fibrils that reduce the functional diameter of the tubule to dimensions that are smaller than most microorganisms. It has been reported that as many as 65% of dentinal tubules in occlusal coronal dentin contain intraluminal collagen fibrils (Dai et al). These intraluminal fibrils act as a net that traps suspended bacteria as fluid flows through the tubules (Fig 1-10). The long-term effects of having bacteria trapped in the tubules depend upon their source of nutrition and the effects of immunoglobulins from the pulp.

## Changes in dentin structure with depth

The area occupied by the lumens of dentinal tubules may be calculated as the product of the cross-sectional area of a single tubule, $\pi r^2$, and $N$, the number of tubules per $cm^2$, where $r$ is the radius of the tubule. Because both the radius of dentinal tubules and their number per unit area increase as one examines dentin from the DEJ to the pulp (Harrán Ponce et al), the area occupied by tubule lumens also increases. Because this area is occupied by dentinal fluid, which is 95% water, it is approximately equivalent to the tubular water content. That is, whereas the water content of dentin near the DEJ is about 1% by volume, it is about 22% by volume close to the pulp.

In terms of clinical significance, the high water content of deep dentin creates problems when bonding to dentin is performed via the etch-and-rinse technique. Upon removal of the smear plugs that block the tubular orifices, water transuded from the dentinal tubules competes with resin monomers for the surfaces of the demineralized collagen fibrils (Pashley et al 2011). This may also result in the entrapment of water blisters within the resin-dentin interface (Fig 1-11). These water blisters act as stress raisers that affect the integrity of the bonded interfaces (Tay et al). Fortunately, water content in nonvital dentin can be controlled, which facilitates bonding to endodontically treated dentin. This phenomenon may have more significance when adhesive resins are used for bonding to dentin. It is well known that loss or leakage of the temporary restorative material at the access opening

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can lead to bacterial contamination of root canal fillings (Kameyama et al). Several studies recommend the use of a secondary seal on the floor of the pulp chamber that extends over the orifice of the filled canal to avoid contamination (Schwartz and Fransman). This not only protects the root canal filling from leakage but also seals off any accessory canals that might exist on the pulpal floor.

## Chemical composition of dentin

The composition of bulk dentin by weight is reported to be 70% mineral, 20% organic, and 10% water (Goldberg et al). Because dentin is highly dense (2.05–2.30 g/cm$^3$), the percentage by weight is much higher than the percentage by volume.

The mineral phase of dentin is a calcium-deficient, carbonate-rich apatite. The carbonated apatite exists as plate-like crystallites in intertubular dentin. Each crystallite is 50 to 60 nm long, 36.4 ± 1.5 nm wide, and 10.3 ± 0.3 nm thick. This small size, relative to large apatite crystals in enamel, is thought to account for the high (6.7) critical pH of dentin. Crystal size is clinically significant because radicular dentin demineralizes at less than 10% of the hydrogen ion concentration required for the demineralization of enamel (pH 5.5). This renders dentin more susceptible to caries than enamel is once root surfaces have become exposed (Hoppenbrouwers et al). About 90% of the organic portion of the dentin matrix is made up of type 1 collagen, with noncollagenous protein growth factors and proteoglycans making up the remaining 10% (Goldberg et al, Bertassoni et al 2012a, Moradian-Oldak and George). The water content of dentin varies according to location, but bulk dentin has been reported to be between 8% and 16% water, most of which is unbound and can be removed by heating the dentin to 120°C. A small fraction of the water (probably less than 1%) is associated with apatite crystallites and with collagen (Agee et al).

## Physical characteristics of dentin

Dentin is a heterogeneous composite material in which the dentinal tubules are surrounded by highly mineralized peritubular dentin (about 95% mineral phase by volume). This peritubular dentin is embedded within considerably less mineralized intertubular dentin (about 30% mineral phase by volume) (Marshall et al).

The atomic force microscope has been used to measure the nanohardness and modulus of elasticity of intertubular and peritubular dentin (Kinney et al 1996, Angker and Swain, Zhang et al). There is wide variation in actual values depending on the location and method in which the stiffness is measured, but one study has reported dentin's modulus of elasticity to be 24.8 ± 1.4 GPa and enamel's to be 95.6 ± 4.9 GPa (Fong et al). The greater elasticity of dentin makes it tougher than enamel, which accounts for its stress-breaking/shock-absorbing function (Seyedkavoosi and Sevostianov).

Dentin is also much softer than enamel (Vickers hardness: 65.6 and 274.8 kg/mm$^2$, respectively; Chun et al), making it more susceptible to wear and permitting efficient endodontic instrumentation with hand files or rotary nickel-titanium instruments. Data derived from nanoindentation indicate that peritubular dentin has a greater nanohardness than intertubular dentin (250 kg/mm$^2$ and 52 kg/mm$^2$, respectively). In addition, deep dentin tends to be more elastic than superficial dentin (with a modulus of elasticity of about 17 vs 21 GPa). The ultimate tensile strength of human mineralized radicular dentin has been reported to vary between 63 and 96 MPa, while that of demineralized radicular dentin varies between 16 and 29 MPa, being stronger when stressed perpendicular to the tubules (Sano et al, Nishitani et al).

Because of the wide variation in the microscopic structure of dentin, one would expect its mechanical properties to vary across regions. Indeed, values for the bulk mechanical properties of dentin are generally listed as a range because they are not uniform. For instance, microhardness values in the crown fall from 350 kg/mm$^2$ in enamel to 50 kg/mm$^2$ in the dentin just beneath the DEJ, then rise again to 70 kg/mm$^2$ within 1 mm of the DEJ before slowly declining to the pulp (40 to 50 kg/mm$^2$ near the pulp) (Wang and Weiner). Similar distributions are found for the modulus of elasticity (Meredith et al). Mantle dentin and the zone of interglobular dentin about 200 μm from the DEJ are less mineralized and less stiff than the inner core of dentin. Radicular dentin has an even lower microhardness, about 30 kg/mm$^2$ at the cementoenamel junction (CEJ), rising to 52 kg/mm$^2$ about halfway across, and then falling to 40 to 45 kg/mm$^2$ near the pulp (Wang and Weiner). This variation in the mechanical properties of dentin plays a critical role in the distribution of mechanical forces throughout a tooth.

# Fractures and Cracks in Dentin

Tooth fractures are encountered by dentists on a daily basis. The outcomes of tooth fracture may be so insignificant that no treatment is required or so severe that root canal treatment or tooth extraction is needed. One form of incomplete tooth fracture, cracked tooth syndrome, often originates from microcracks in the dentin and presents a diagnostic challenge (Gill et al). Another form of incomplete root fracture, vertical root fracture, is a longitudinally oriented fracture of the root initiating at the apex and propagating to the coronal part of the root (Khasnis et al).


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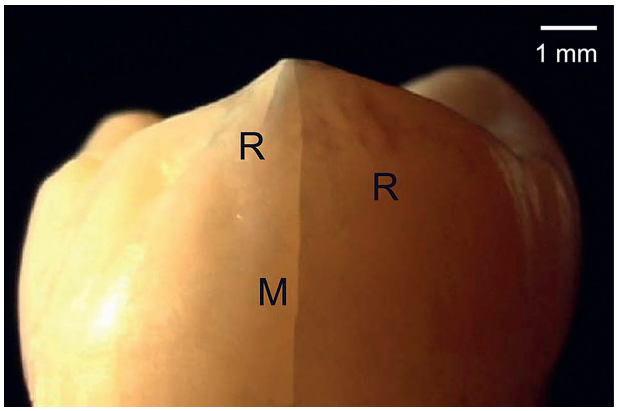
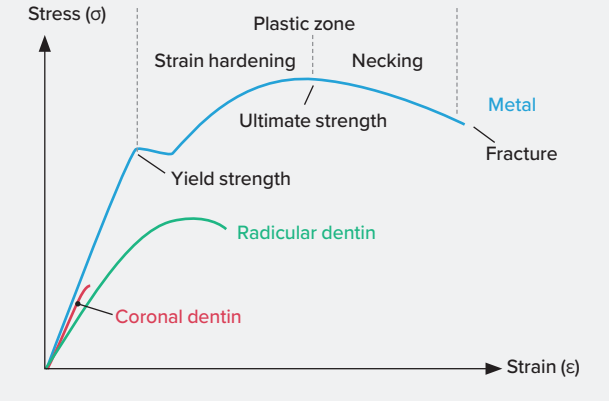



▲ **FIG 1-12** Crack formation on a human molar after contact with a tungsten carbide indenter with flat end on top of the cusp. M = margin crack; R = radial crack. (Reprinted with permission from Chai et al.)




▲ **FIG 1-13** Representative stress-strain curves for ductile metal *(blue)*, coronal dentin *(red)*, and radicular dentin *(green)*. (Redrawn after Chen et al with permission.)

Fracture, secondary caries, and marginal deterioration are the three predominant forms of restorative tooth failure (Ivancik and Arola). Vertical root fracture specifically is the third most common reason for the extraction of a root-treated tooth. Tooth fracture occurs through a process that involves fatigue and fatigue crack growth (Yahyazadehfar et al). Because of the cyclic loading that occurs during mastication and the potential for flaws to be introduced within the tooth crown during restorative procedures and within the radicular dentin during root canal treatment, the fatigue and fracture properties of dentin are critically important (Ivancik et al). Understanding variations in the fracture toughness of dentin in human teeth at different ages is important for performing successful restorative dentistry and root canal treatment.

Two types of cracks may develop in teeth under contact loading: longitudinal cracks and margin cracks. Longitudinal cracks are subcategorized into radial cracks and median cracks (Chai et al; Fig 1-12). Radial cracks initiate from preexisting flaws at the base of the enamel and extend upward to the occlusal surface. Median cracks commence from shear-nucleated flaws within the quasiplastic yield zone directly beneath the contact load at the occlusal surface and then extend downward through the enamel. Margin cracks initiate from intrinsic defects (eg, protein-rich tufts) at the DEJ and propagate to the occlusal surface. Additional loading causes other modes of failure, such as chipping, cone cracking outside the contact zone, delamination failure at the DEJ, and progression of the crack into the dentin.

## Fracture properties of dentin

Tooth fracture is more likely to originate from cracks that initiate and propagate within dentin than from cracks in the tooth surface. In materials science, fracture toughness is the critical stress intensity factor of a sharp crack, where propagation of the crack suddenly becomes rapid, unlimited, and ultimately catastrophic. Fracture toughness varies by approximately four orders of magnitude across materials. Cracks do not easily propagate in tough materials. Metals, for example, have the highest fracture toughness values and are highly resistant to cracking under stress. Their stress-strain curve exhibits a large zone of plastic flow (Fig 1-13). Compared to metals, dentin is much less tough, but it is considerably tougher than enamel.

An important aspect of the fracture properties of dentin is its tubule orientation. For cracks extending parallel and perpendicular to dentinal tubules, the fracture toughness (KIc) of dentin is estimated to be approximately 2.0 MPa•$m^{0.5}$ and 1.1 MPa•$m^{0.5}$, respectively (Iwamoto and Ruse). For comparison, the KIc value of titanium alloy is 84 to 107 MPa•$m^{0.5}$ (AZO Materials). Hence, one can appreciate why cracked tooth syndrome and vertical root fracture are manifested more frequently than expected.

Because of variations in its microstructure, the fracture resistance of human dentin is location dependent. For example, the fracture toughness of radicular dentin (4.8 MPa•$m^{0.5}$) is substantially higher than that of coronal dentin (2.1 MPa•$m^{0.5}$) (Wang). This difference in fracture resistance is related to the orientation of collagen fibrils in the coronal and radicular dentin (Yahyazadehfar et al). Likewise, the fracture toughness of inner dentin (2.2 ± 0.5 MPa•$m^{0.5}$) is significantly lower than that of midcoronal dentin (2.7 ± 0.2 MPa•$m^{0.5}$) and outer dentin (3.4 ± 0.3 MPa•$m^{0.5}$) (Ivancik and Arola). In comparison, the facture toughness of inner and outer enamel is 1.1 to 3.9 MPa•$m^{0.5}$ and 0.7 MPa•$m^{0.5}$, respectively (Bajaj and Arola). Accordingly, dentin is more resistant to crack propagation than enamel.

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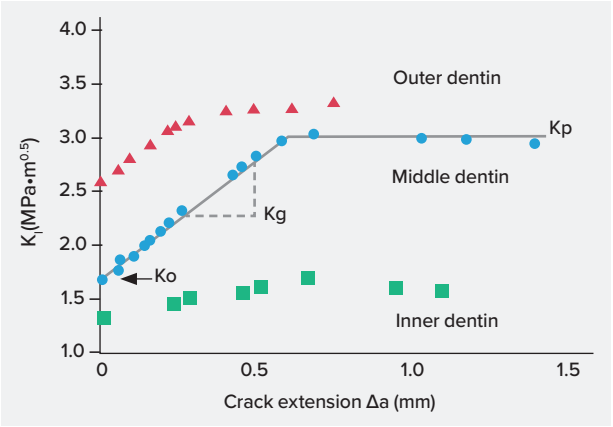


◀ **FIG 1-14** Representative R-curves for young dentin. Ko = initiation toughness; Kg = growth toughness; Kp = plateau toughness derived from a single R-curve. (Redrawn after Chen et al with permission and courtesy of Dr Dwayne Arola.)

## Crack toughening mechanisms in dentin

Dentin increases in toughness in response to crack propagation, as can be seen in the typical crack growth resistance curves (R-curves) of dentin (Ivancik and Arola; Fig 1-14). Dentin is more brittle than most metals. The irreversible work associated with plastic deformation in metal is considerably less prominent in dentin. Hence, the mechanisms that improve the toughness of dentin differ from those for metals. Different toughening mechanisms are responsible for the increasing resistance of a brittle material against crack propagation. They may be classified into intrinsic and extrinsic toughening mechanisms (Ritchie).

Intrinsic toughening mechanisms operate in front of the crack tip to enhance the material's inherent resistance to microstructural damage and cracking. Examples of these mechanisms are the inclusion of hidden length in sacrificial bonds, microcracking, fibrillar sliding, and molecular uncoiling. In dentin, the collagen molecules within the fibrils of the dentin collagen matrix have a quarter-staggered arrangement that enable the molecules to slide against one another during stretching (Tang et al). In addition, these closely packed collagen molecules are bonded by only weak electrostatic or hydrophobic interactions that may break and reform to facilitate energy dissipation during dynamic fibril deformation (Streeter and de Leeuw). This ability to dissipate energy enables the formation of plastic zones around newly emerging cracks, which facilitates crack tip blunting. These mechanisms occur at the scale of tens to hundreds of nanometers and create plasticity that is responsible for the intrinsic toughness of dentin.

On a larger scale, the fracture tolerance of dentin relies on extrinsic toughening mechanisms that inhibit crack growth. These mechanisms operate behind the crack tip by promoting crack tip shielding. The major contribution of these mechanisms is dependent upon the crack path and how it interacts with the dentin microstructure. Two important extrinsic toughening mechanisms have been identified in dentin, namely crack bridging and crack deflection (Kruzic et al, Ivancik et al).

Crack bridging occurs in the wake behind a crack front, where bridging ligaments between microcracks connect the crack faces. By adding extra pressure during the crack opening, these bridging elements decrease the stress intensity near the crack tip and toughen the material. An example of ligaments bridging the crack and microcracks observed during crack growth in outer dentin is shown in Fig 1-15 (Ivancik and Arola). Crack bridging is the major toughening mechanism in dentin and accounts for 26% of the total energy required for fracture in inner, middle, and outer dentin. In young dentin, the linkage of a large fraction of microcracks to the main crack tip may result in regions of intact, unbroken dentin that act as crack-bridging ligaments (Koester et al). The contribution of extrinsic toughening is 2.5 times greater in the outer dentin than in the inner dentin (Ivancik and Arola).

Crack deflection occurs when a crack path deviates from the plane of maximum driving force because of features present in the microstructure of a material. Crack deflection is frequently encountered in polycrystalline ceramics or composite materials that contain hard phases, where the crack propagates intergranularly. In dentin, dentinal tubules are the most obvious features that cause crack deflection. This toughening mechanism becomes important when the growing crack is oriented perpendicular to the dentinal tubules. In such a scenario, a propagating crack tip makes small turns and twists along the axis of the dentinal tubules (Koester et al; Fig 1-16). Crack deflection reduces the stress intensity experienced at the crack tip and provides an additional source of extrinsic toughening.

Empty dentinal tubules in human dentin enhance fatigue resistance by blunting crack tips (Arola and Reprogel). When a propagating crack penetrates a tubule, the radius of the crack tip increases from near atomistic dimensions to the radius of the tubule. This markedly reduces the stress field of the crack tip. This toughening mechanism,


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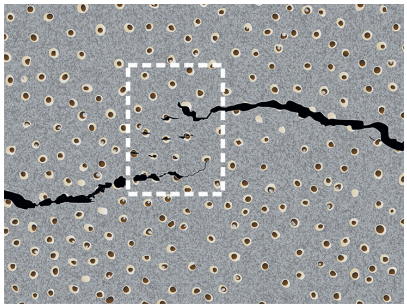
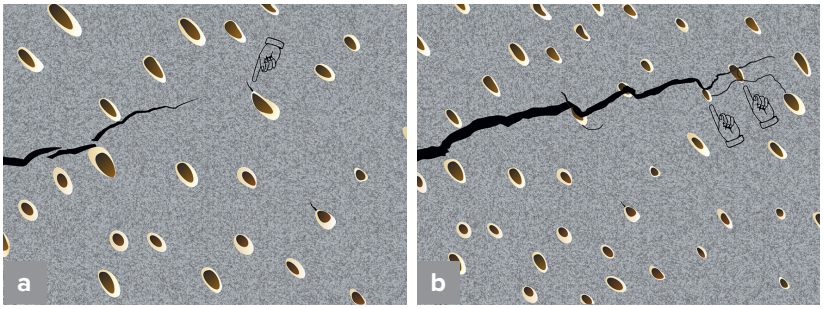


▲ **FIG 1-15** Crack bridging in dentin. A ligament (ie, an unbroken portion of dentin; *white dotted box*) bridges a crack, and microcracks are visible during crack growth in the outer dentin. (Reprinted with permission from Chen et al.)




▲ **FIG 1-16** Crack deflection and crack branching. A growing crack in dentin illustrates how unfilled dentinal tubules interact with a growing crack. *(a)* Initial crack before loading. *(b)* The propagating crack extends through the microcracks associated with a dentinal tubule to form deflections and branching in the crack front. The *pointers* indicate regions where the growth of microcracks and crack branching occur during crack growth. (Reprinted with permission from Chen et al.)

however, does not occur in filled dentinal tubules. Cracked dentinal tubules can also contribute to toughening dentin by promoting crack branching (see Fig 1-16). Branched cracks enable distribution of the overall stress intensity across the multiple crack tips (Koester et al).

Although less prominent than in metals, inelastic (plastic) deformation does play a role in toughening dentin, particularly radicular dentin. This can be observed in a typical tensile stress-strain curve of radicular dentin (see Fig 1-13). The linear stress-strain stage is followed by a nonlinear, inelastic stage. Plastic deformation in dentin occurs via microcracking. Structural elements in dentin, such as dentinal tubules, peritubular dentin, and the incremental lines, directly contribute to the development of microcracks (Eltit et al). Nevertheless, the toughening effect of microcracks in dentin is secondary to that of crack bridging (Nalla et al). From a macroscopic perspective, the double-layer structure of dentin (the more brittle inner peritubular dentin and the more resilient outer intertubular dentin) is also responsible for fracture resistance (An and Zhang).

## Cracks in coronal dentin

Fracture toughness increases from the pulpal (inner) side to the DEJ (outer) side of coronal dentin, which can be attributed to site-specific crack growth toughening. The average tubule density in the inner, middle, and outer dentin is 48,500 ± 9,800; 29,200 ± 2,800; and 19,700 ± 3,700 tubules/mm$^2$, respectively, and the difference in toughening behavior in these three locations is an indirect reflection of the tubule density.

In inner dentin, the tubule lumens are highly influential in the development of the crack path and the amount of energy needed for crack growth. Cracks within the inner dentin propagate in the direction of the maximum opening stress but with preference for the nearest lumen adjacent to the crack tip. Toughening is achieved when cracks meander to adjacent lumens and unbroken ligaments develop bridging between the cracks (Ivancik and Arola). In middle dentin, the tubules are separated from intertubular dentin by a thin layer (0.5-μm thick) of highly mineralized peritubular dentin with a relatively large elastic modulus (An and Zhang). This peritubular cuff is an important microstructural parameter that contributes to dentin toughness. Microcracking of the peritubular cuff spurs the development of satellite cracks. These satellite cracks eventually merge with the main crack and facilitate the development of unbroken ligaments that create crack closure. Crack growth within the outer dentin is accompanied by extensive bridging by unbroken ligaments. This mechanism represents the largest extrinsic component of crack growth toughening.

## Cracks in radicular dentin

Vertical root fractures (Fig 1-17) present the greatest challenge to general dentists and endodontists because it is difficult to obtain a clear diagnosis, even with the aid of CBCT (Saberi et al). Also, treatment options for vertical root fractures are limited (Liao et al). The damage tolerance of radicular dentin deteriorates with age. This degradation is most severe near the root apex and appears to result from changes in the microstructure and chemical composition of radicular dentin, as well as an increase in collagen cross-linking (Yan et al 2017).

Radicular dentin has lower lumen density and reduced peritubular cuff thickness compared to coronal dentin. Both these features make it more akin to outer coronal dentin and would be expected to result in greater toughening. Moreover, collagen fibrils in radicular dentin have larger diameters and a different primary orientation than in the crown. These morphologic features also contribute to greater toughening in radicular dentin (Ivancik and Arola).

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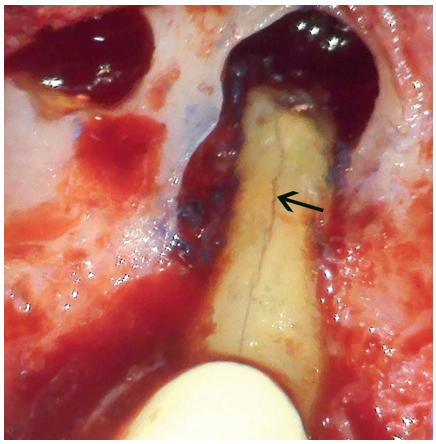

▲ **FIG 1-17** Vertical root fracture *(arrow)* in the root of a maxillary right canine that could not be detected via periapical radiograph or CBCT. The fracture line became apparent only after apicoectomy and methylene blue staining. (Photograph courtesy of Dr Kevin Jett; reprinted with permission from Chen et al.)

Compared with coronal dentin, radicular dentin is highly anisotropic in fracture behavior, with planes defined by the incremental lines exhibiting the lowest fracture resistance (Saberi et al). In coronal dentin, cracks along and perpendicular to the dentinal tubules compete with the incremental lines, resulting in more isotropic fracture behavior. The root has higher fracture energy than the crown, which can be seen as the area under the stress-strain (load-deflection) curves of coronal dentin versus radicular dentin (see Fig 1-13). The more brittle behavior of coronal dentin is due to the thicker peritubular cuffs.

Collagen fibrils in radicular dentin are predominantly oriented in a root-crown direction along the incremental planes formed during dentinogenesis. This is not the case in coronal dentin, where the collagen fibrils are laid down randomly on the incremental planes. Differences in the organization of collagen fibrils may also account for the difference in fracture toughness between radicular dentin and coronal dentin (Wang).

## Why does tooth fracture occur?

In light of the various mechanisms available for toughening dentin, one is compelled to ask: Why does tooth fracture occur so frequently? Tooth fracture is a multifactorial problem, and it is very difficult to identify its cause. The most direct answer is that the fracture toughness values of dentin (ie, plateau toughness [Kp in Fig 1-14]) relative to other materials are low (~2.5 $MPa \cdot m^{0.5}$). These values are at least 10 times lower than the fracture toughness of most engineering materials. More importantly, the initiation toughness of dentin (Ko in Fig 1-14) is very low (~1 $MPa \cdot m^{0.5}$). This allows cracks to initiate from incipient damage in teeth under low stress (eg, biting on a bone chip or a fruit pit, root canal instrumentation with rotary instruments). Although dentin exhibits some interesting toughening mechanisms that allow it to resist some crack growth, these mechanisms are often inadequate.

There are also other issues to consider. Degradation of dentin toughness invariably occurs in the growth toughness region (Kg in Fig 1-14). The literature suggests that vertical root fractures and cracked tooth syndrome predominately occur in restored teeth. Restorations can create residual stresses that reside in the tooth as a static component. The presence of static stresses can exhaust a tooth of its potential for energy dissipation. This, in turn, embrittles the tooth and reduces its toughness. With cyclic loading (as in the case of mastication), crack growth proceeds with much less resistance. Other important factors are related to age (see the following section) and whether root canal treatment has been performed. Long-term intracanal medication and retreatment are more likely to cause vertical root fracture (García-Guerrero et al). The contribution of root canal treatment to vertical root fracture is beyond the scope of this chapter.

## Effects of aging on the fracture resistance of dentin

There is general consensus that the incidence of cracked teeth increases with age (Roh and Lee, Yang et al, Aboud et al). In a demographic examination of 227 teeth, almost 90% of vertical root fractures occurred in patients over 40 years of age (Cohen et al). This raises concerns about the reduction of fracture resistance in oral hard tissues (especially dentin) with age. Studies on coronal dentin report a substantial reduction in fracture toughness (Koester et al, Nazari et al) and fatigue crack growth resistance (Bajaj et al, Ivancik et al) with aging. For example, the average resistance to crack growth for young dentin (between 18 and 35 years old) was characterized by an initiation toughness (Ko) of 1.34 $MPa \cdot m^{0.5}$ and a plateau toughness (Kp) of 1.65 $MPa \cdot m^{0.5}$. In old dentin (> 55 years old), the Ko (1.08 $MPa \cdot m^{0.5}$) and Kp (1.17 $MPa \cdot m^{0.5}$) values were significantly less than in young dentin (Bajaj et al).

The age-associated deterioration of dentin's mechanical properties is attributed to two factors:

1. Changes in the chemical composition of intertubular dentin
2. Changes in the extent of tubular occlusion

In young dentin, toughening results from a combination of inelastic deformation of the mineralized collagen


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matrix and microcracking of the peritubular cuffs. In the former process, the less-cross-linked young dentin allows collagen molecules to slide within the mineralized fibrils to dissipate stress. In the latter process, the presence of open, unfilled tubules enables the formation of microcracks. These microstructural changes facilitate further toughening of the young dentin via the development of unbroken ligaments of dentin and posterior crack bridging (Bajaj et al).

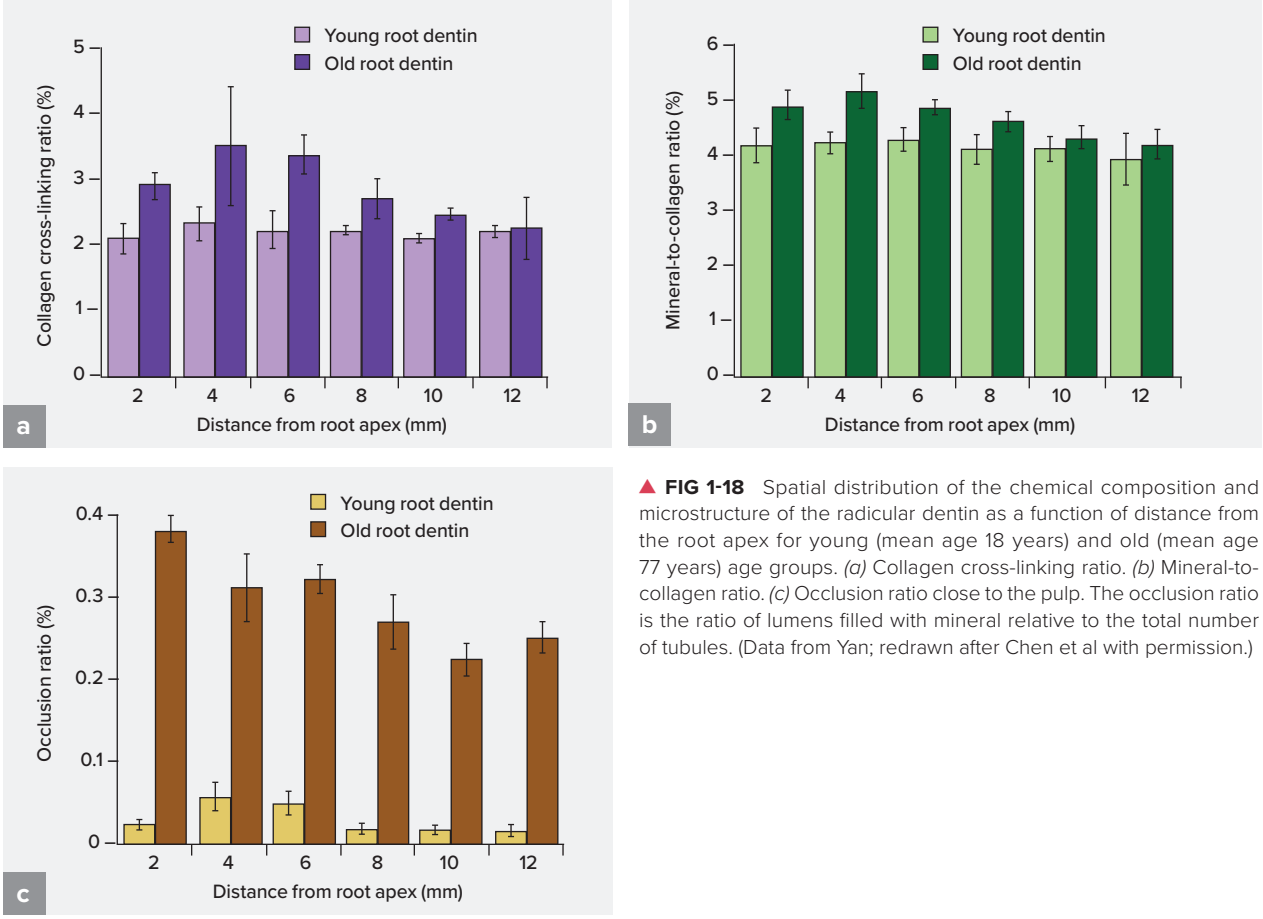
Both intra- and interfibrillar collagen cross-linking increase with age (Walters and Eyre, Martin-de las Heras et al), resulting in increases in the collagen cross-linking ratio in old dentin (Yan; Fig 1-18a). In addition, there are higher levels of advanced glycation end products resulting from the deterioration of the collagen matrix in aged dentin (Shinno et al). Collagen deterioration also increases the mineral-to-collagen ratio (Yan; Fig 1-18b), which reduces the capacity for near-tip inelastic deformation in old dentin.

With respect to tubular occlusion, age decreases patent dentinal tubule area in radicular dentin due to sclerosis (Vasiliadis et al). This results in a significant increase in the dentinal tubule occlusion ratio (Yan; Fig 1-18c). As mentioned previously, the presence of filled dentinal tubules decreases the capacity for microcracking. This, in turn, suppresses the formation of unbroken ligaments and the degree of extrinsic toughening.

## Dentin Permeability

### Permeability of coronal dentin

Dentin can be regarded as both a barrier and a permeable structure, depending on its thickness, age, and other variables (Pashley et al 2002, Mjör 2009). Once exposed due




▲ **FIG 1-18** Spatial distribution of the chemical composition and microstructure of the radicular dentin as a function of distance from the root apex for young (mean age 18 years) and old (mean age 77 years) age groups. *(a)* Collagen cross-linking ratio. *(b)* Mineral-to-collagen ratio. *(c)* Occlusion ratio close to the pulp. The occlusion ratio is the ratio of lumens filled with mineral relative to the total number of tubules. (Data from Yan; redrawn after Chen et al with permission.)

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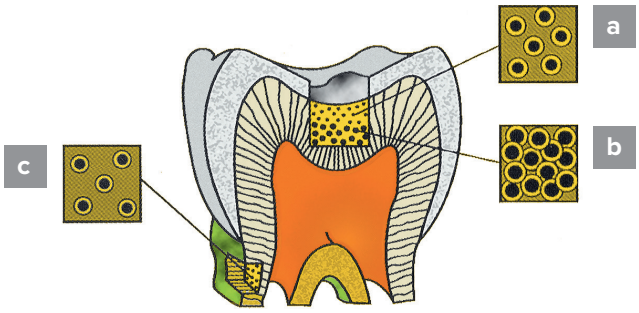
to trauma or tooth preparation, dentinal tubules become diffusion channels for the passage of solutes and solvents across dentin. Fluid flow in dentin demonstrates similarities to a porous media in which liquid flows through an assembly of channels of various shapes and diameters. The ease with which solutes diffuse inward across dentin down a chemical gradient may be expressed by the *permeability coefficient*, a term adopted by Pashley to describe the inward diffusion of different molecules across dentin (Pashley 1990):

$$\textit{Permeability coefficient} = DA\,\frac{\Delta C}{\Delta X}$$

where $\Delta C$ is the change in solute concentration, $\Delta X$ is the dentin thickness, $A$ is the dentinal tubule area, and $D$ is the diffusion coefficient.

The intrinsic capacity of solutes to diffuse across dentin is inversely proportional to the cube root of their molecular weight (Pashley 1990). The permeability of dentin is highest for small molecules, such as water; lower for larger molecules, such as albumin and immunoglobulins; and still lower for molecules with molecular weights over 106, such as endotoxins (Pashley and Livingston). Because both the density and diameter of dentinal tubules increase with dentin depth from the DEJ to the pulp (Fig 1-19), the permeability of dentin is lowest at the DEJ and highest at the pulp. Hence, it is easier for the same type of solute to diffuse across deep dentin than shallow dentin (Fig 1-20). At any depth, however, the permeability of dentin in vitro is far below what would be predicted by the actual density and diameters of the dentinal tubules. This is because of intratubular materials, such as collagen fibrils, and the mineralized constrictions of the tubules.

Dentin permeability is divided into two broad categories: *(1)* transdentinal movement of substances through dentinal tubules, such as fluid shifts in response to hydrodynamic stimuli, and *(2)* intradentinal movement of exogenous substances into intertubular dentin, as occurs with the infiltration of hydrophilic adhesive resins into demineralized dentin surfaces during resin bonding (Fig 1-21a). In exposed vital dentin with open tubules, the inward diffusional flux of materials competes with the rinsing action of outward convective fluid transport (Pashley and Matthews, Charoenlarp et al; Fig 1-21b). This mitigates the inward flux of potentially irritating bacterial products into exposed, sensitive dentin. The hydraulic conductance of a




◀ **FIG 1-19** *(a and b)* Cavity prepared on the occlusal surface of coronal dentin. *(c)* Cavity prepared at the cervical region of the root surface. Note that there are fewer tubules in superficial dentin *(a)* than in deep dentin *(b)*. The dentinal tubules are not only closer together in deep dentin but also larger in diameter. This combination is responsible for the exponential increase in dentin permeability with depth. (Adapted from Pashley 1996.)

▲ **FIG 1-20** Dentin thickness is one of the factors that affects the diffusion of noxious substances into the pulp via the dentinal tubules. According to the permeability coefficient formula, the permeability of deep dentin (eg, dentin 0.1 mm from the pulp) is much higher than the permeability of shallow dentin near the DEJ (eg, dentin 2.5 mm from the pulp).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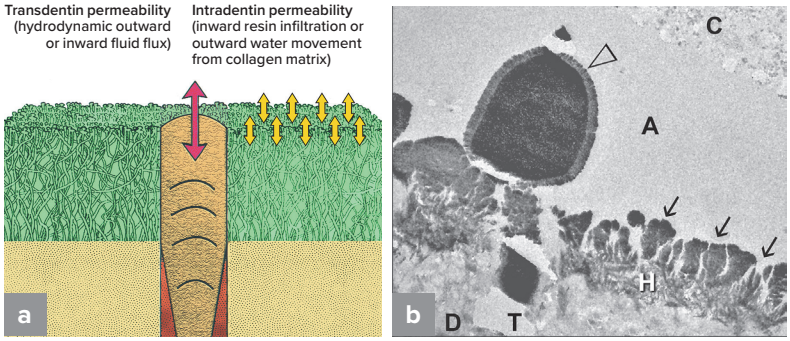
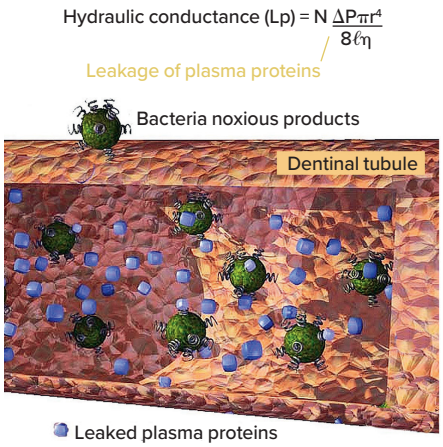
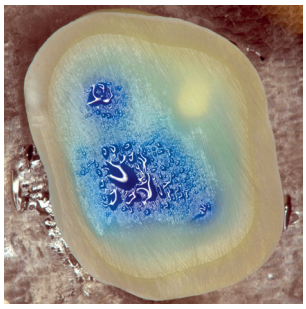


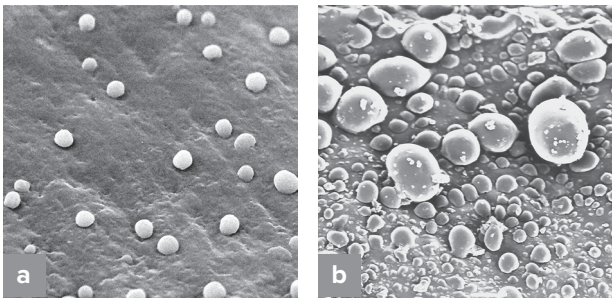

▲ **FIG 1-21** *(a)* Contrast between transdentinal permeability due to the inward or outward movement of dentinal fluid or materials within tubules and intradentin permeability, in which materials or water diffuse in or out of porosities between tubules resulting from demineralization. *(b)* Transmission electron micrograph of a silver-impregnated interface of coronal dentin bonded with a permeable one-step self-etch adhesive under physiologic pulpal pressure of 15 $cmH_2O$. This micrograph illustrates both the outward transdentinal permeation of a water droplet *(open arrowhead)* into the adhesive layer (A) and the outward intradentin permeation of water *(arrows)* from the demineralized collagen matrix that is partially infiltrated by the adhesive (ie, the hybrid layer). The latter type of water movement has been described as *water trees*. C = composite; D = dentin; H = hybrid layer; T = dentinal tubule.


Hydraulic conductance (Lp) = N ΔPπr⁴ / 8ℓη
Leakage of plasma proteins
Bacteria noxious products
Dentinal tubule
Leaked plasma proteins

▲ **FIG 1-22** Effect of the viscosity of dentinal fluid on hydraulic conductance (Lp). Because *Lp* is inversely proportional to the eighth power of viscosity, the leakage of plasma proteins (eg, fibrinogen, albumin) will reduce the movement of noxious bacteria products through the dentinal tubules into the pulp.



◀ **FIG 1-23** Photograph showing dye fluid moving across the dentin from the underlying pulp chamber in vitro under a simulated pulpal pressure of 20 $cmH_2O$. Note that the dentin is most permeable over the pulp horns, where the tubules have their largest diameters and densities.




▲ **FIG 1-24** Epoxy resin replicas of dentin surfaces showing the transudation of dentinal fluid from vital, instrumented human dentin. *(a)* Dentin permeability is low when vital dentin is covered with a smear layer. *(b)* Higher permeability is observed after removal of the smear layer.

tissue provides information about the ease with which a fluid can move across a unit of surface area under a unit of pressure per a unit of time. The measurement of hydraulic conductance in vitro provides information about the factors that determine tubular fluid movement and dentin permeability. Hydraulic conductance (*Lp*) is expressed as:

$$Lp = N \frac{\Delta P \pi r^4}{8 l \eta}$$

where *Lp* is hydraulic conductance in $\mu L \cdot cm^{-2} \cdot min^{-1} \cdot cmH_2O^{-1}$, $\Delta P$ is the pressure difference across dentin, *N* is the number of dentinal tubules/$cm^2$, *r* is the radius of the dentinal tubules, and $\eta$ is the fluid viscosity.

As an example of the application of this formula, an increase in fluid viscosity caused by the leakage of plasma proteins from pulpal blood vessels into the dentinal fluid of vital deep dentin would result in a large decrease in *Lp*. This is because *Lp* is inversely proportion to the eighth power of fluid viscosity (Fig 1-22).

The permeability of dentin varies widely, especially on occlusal surfaces, where perhaps only 30% of dentinal tubules (Fig 1-23) are in free communication with the pulp (Pashley et al). Axial dentin is much more permeable than occlusal dentin (Pashley and Pashley, Maroli et al). The gingival floor proximal boxes and the gingival extension of finish lines in crown preparations often end in highly permeable regions of dentin (Garberoglio). As many as 4 million exposed dentinal tubules are found on a surface area of approximately 1 $cm^2$ of full-crown preparations on posterior teeth (Pashley 1991, Richardson et al). Although dentinal tubules are occluded by smear layers and/or cement following the placement of castings, both cement and smear layers have finite solubility, and some tubules may become exposed over time, most likely at

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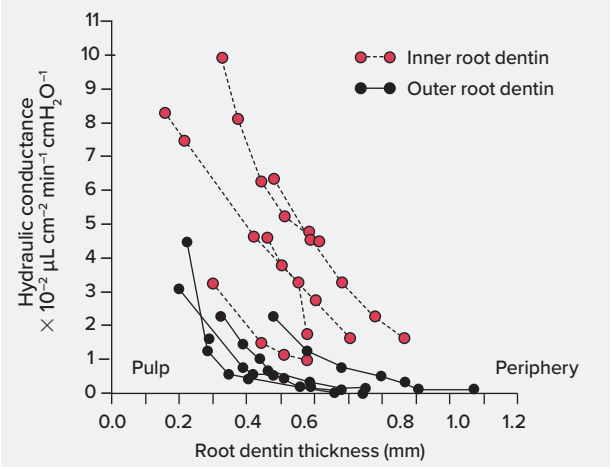
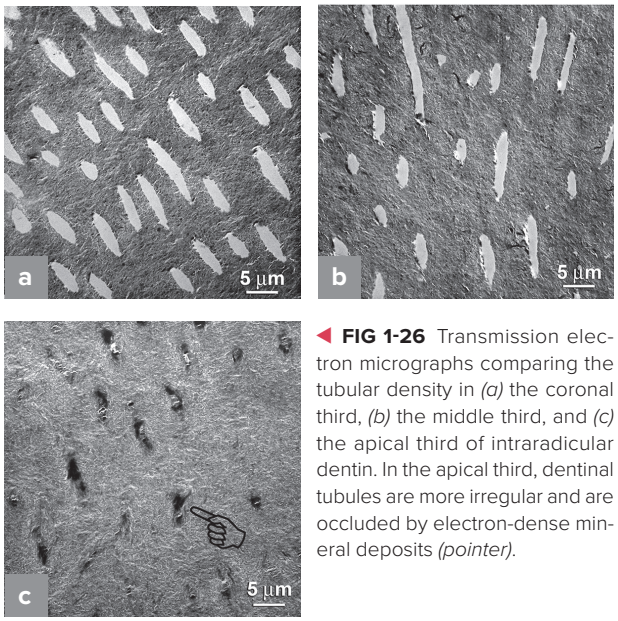


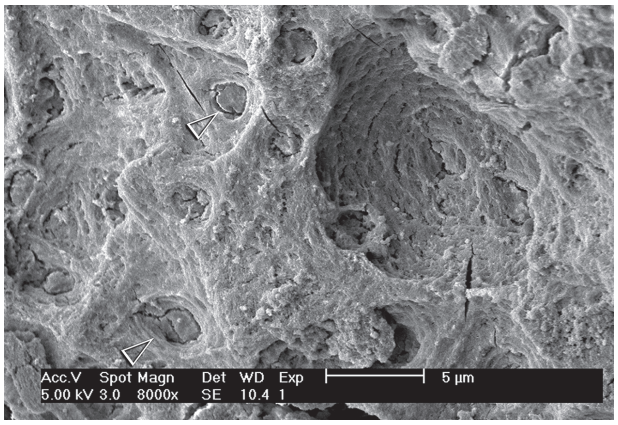

▲ **FIG 1-25** Permeability of inner and outer radicular dentin as a function of dentin thickness. Flat root surfaces were split longitudinally into outer and inner halves, each about 1.1 mm thick. The permeability of the outer half was low (from 1.1 to 0.9 mm) even after the removal of cementum, and permeability increased slowly only as the dentin was made thinner by grinding down the periphery. The rate of increase in radicular dentin permeability was much higher in inner dentin. (Data from Fogel et al.)




◀ **FIG 1-26** Transmission electron micrographs comparing the tubular density in *(a)* the coronal third, *(b)* the middle third, and *(c)* the apical third of intraradicular dentin. In the apical third, dentinal tubules are more irregular and are occluded by electron-dense mineral deposits *(pointer)*.

the most peripheral extensions of the restoration, where diffusion distances to the pulp are shortest (Fig 1-24).

The permeability of sclerotic dentin is very low, regardless of whether the sclerosis is due to physiologic or pathologic processes (Tagami et al, Senawongse et al 2008). This is because the tubules become filled with mineral deposits (Tay and Pashley, Kinney et al 2005, Porter et al). This reaction is fortuitous in that it slows the caries process and tends to protect the pulp (Zavgorodniy et al). Most pulpal reactions to cavity preparations or restorative materials used on carious dentin are due to changes in the adjacent normal dentin rather than in the almost-impermeable caries-affected dentin (Yoshihara et al).




▲ **FIG 1-27** The apical third of an instrumented and sodium hypochlorite–irrigated root canal shows completely obliterated dentinal tubules *(open arrowheads)*. These occluded tubules are not amenable to penetration by bacteria. The cracks in the dentin are drying artifacts. The large circular structure is a mineralized lateral canal.

## Permeability of radicular dentin

As with coronal dentin, the permeability of radicular dentin depends upon dentin thickness, the number of tubules/mm$^2$, and the diameter and patency of the dentinal tubules (Pashley 1991). There are more tubules/mm$^2$ in deep dentin near the canal than in peripheral dentin because the outer circumference of the root is greater than the circumference of the root canal (Fig 1-25). The number of tubules (and odontoblasts) remains constant from the outer radicular dentin to the root canal. However, the tubules become crowded together at the canal (Pissiotis and Spångberg). Dentinal tubule density is reduced from the coronal third to the apical third of root canals (Lo Giudice et al; Fig 1-26). Although the permeability of dentin should be proportional to the area occupied by dentinal tubules, quantitative comparisons of the theoretical calculated permeability versus the actual measured permeability revealed the latter to be less than 3% of the theoretical value. This finding is explained by the fact that dentinal tubules are not smooth bore tubes but contain a good deal of intratubular material, such as mineralized inclusions and collagen fibrils. Tubular sclerosis, a physiologic phenomenon that commences in the third decade of life in the apical root region and advances coronally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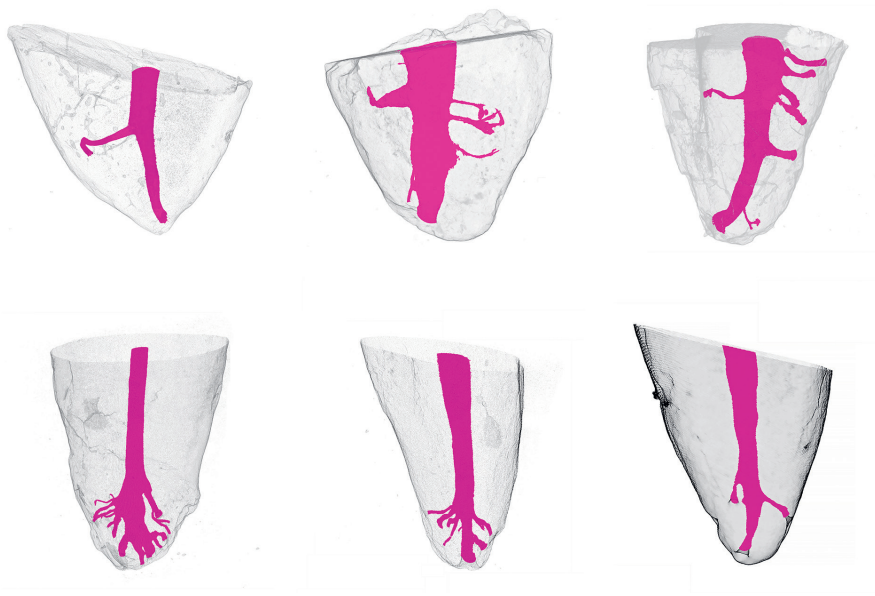

**FIG 1-28** 3D reconstructions of microCT images of the apical 3-mm segments of different root canals showing variations in the arrangement and number of patent accessory canals. (Images courtesy of Professor Bing Fan and Dr Mengting Duan.)

with age, is the main factor influencing the permeability of apical radicular dentin (Carvalho and Lussi; Fig 1-27).

Dentin permeability has important clinical implications. For example, sodium hypochlorite is commonly used as an irrigant in endodontic procedures. Soaking dentin disks in 5% sodium hypochlorite for 1 hour produces a 105% increase in the hydraulic conductance of human cervical dentin (Barbosa et al). In contrast, soaking dentin disks with 35% hydrogen peroxide for 1 hour produces only a 16% decrease in permeability.

The softest layer of inner dentin is removed during instrumentation of the canal space in root canal treatment. The increase in the diameter of the root canal leads to a decrease in the number of tubules/mm$^2$ (the exact opposite of what occurs in operative dentistry when drilling is performed from the enamel toward the pulp). In addition, the radicular dentin is made slightly thinner (Tao et al). These two phenomena tend to influence radicular dentin permeability in opposite ways, with the effects of reduced dentin thickness dominating the effects of increased root canal diameter. Shaping the canal, however, also creates long smear plugs (Mader et al) and thick smear layers (Fogel et al) on the instrumented surface that decrease dentin permeability.

When the hydraulic conductance of coronal and radicular dentin was measured, radicular dentin was found to have a permeability that is only about 3% to 8% that of coronal dentin. The low permeability of radicular dentin prevents sulcular endotoxins from diffusing into the pulp (Nissan et al). The low permeability of the cementum also contributes significantly to the barrier properties of the root (Petelin et al).

The permeability of radicular dentin displays regional differences along its axial length. Cervical and middle radicular dentin are more permeable than apical dentin (Tao et al, Wiebkin et al), a finding that is consistent with their higher tubule density (Lo Giudice et al). The permeability of the pulp chamber floor in the furcation region is reported to be high because of the presence of patent accessory canals (Xu et al, Chouchi et al; Fig 1-28).

Endodontists need to be aware of the tubule density of apical dentin because this region is beveled during root end surgery, during which the root is resected to facilitate placement of a root end filling. Root resection exposes dentinal tubules in the apical part of the root canal and introduces the possibility of tubule leakage. The greater the angle of the root resection is, the greater the potential for microleakage (Gilheany et al). Thus, minimally angled resections have been advocated, along with placement of the reverse filling below the edge of the bevel to prevent leakage through dentinal tubules exposed by the bevel. Theoretically, the deeper the bevel is, the deeper the root end preparation should be prepared to prevent microleakage. Nevertheless, a more recent study reports that the apical resection angle (0 or 45 degrees) does not significantly affect the sealing ability of contemporary root end filling materials (Jain et al). This is probably because tubule density is much lower in apical dentin than in coronal dentin. The diameter of apical dentinal tubules may be further reduced with age. As the odontoblasts in the apical part of the canal space undergo apoptosis, the dentinal tubules undergo sclerosis over time with increased deposition of intratubular mineral crystals (Paqué et al, Yan et al 2022).

## Balance between the permeation of noxious substances across dentin and clearance

There is a balance between the rate at which materials permeate across exposed dentin to reach the pulp and

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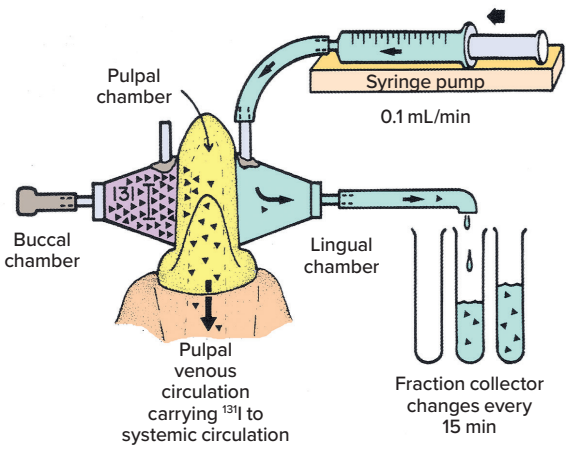
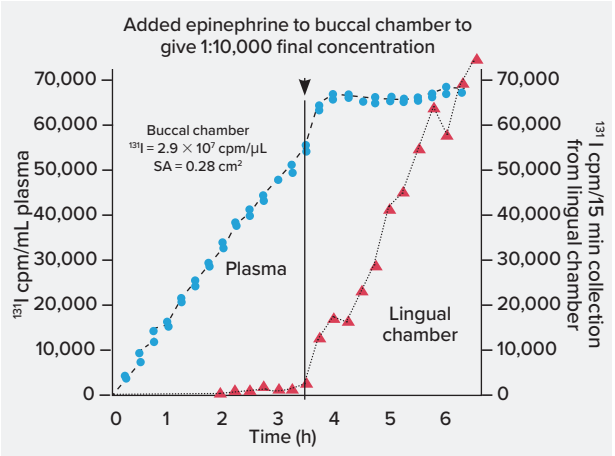



▲ **FIG 1-29** Illustration of how dual chambers were placed on the buccal and lingual surfaces of dog molars to measure both the systemic absorption of substances applied to dentin and changes in the pulpal interstitial fluid concentration of substances. *Triangles* indicate radioactive iodide. (Adapted from Pashley 1979.)




▲ **FIG 1-30** Effects of topical epinephrine on pulpal interstitial fluid iodide concentration. Over the first 3.5 hours, the plasma iodide concentration rose linearly as iodide permeated the buccal dentin into the pulp, where the microcirculation cleared it so efficiently that little iodide ever showed up in rinsings from the lingual chamber. However, as soon as epinephrine was added to the buccal chamber, the pulpal clearance stopped, and the interstitial fluid concentration rose and began diffusing across the lingual dentin into the lingual chamber. (Data from Pashley 1979.)

the rate at which they are cleared from interstitial fluid via pulpal microcirculation and lymphatic vessels (Pashley 1979; see Fig 1-22). This is demonstrated in Fig 1-29, which illustrates what happened when conical chambers were placed on the buccal and lingual surfaces of a dog molar in vivo. Radioactive iodide was placed in the buccal chamber. The lingual chamber was perfused with samples collected by a fraction collector. Blood samples, drawn every 10 minutes, revealed that the pulpal circulation cleared almost all the iodide that permeated the buccal dentin. Little iodide showed up in the rinsing fluid of the lingual chamber until epinephrine was added to the buccal chamber, causing cessation of pulpal blood flow and the subsequent accumulation of iodide in the pulp (Fig 1-30). This was indicated by the large increase in iodide diffusing across the lingual dentin. Procedures that reduce pulpal blood flow (eg, activation of pulpal sympathetic nerves, administration of vasoconstrictor agents with local anesthetics) upset this balance, resulting in higher interstitial fluid concentrations of extrinsic material than would occur at normal levels of capillary flow (Pashley et al 2002).

Interventions that stimulate pulpal blood flow can lead to reduced interstitial levels of materials by reducing the permeability of dentinal tubules and increasing clearance by the pulpal microvasculature. For example, electrical stimulation of the inferior alveolar nerve in cats causes an increase in both pulpal blood flow and the outward movement of dentinal fluid (Vongsavan and Matthews 1992, Matthews and Vongsavan). This shifts the inflow-outflow balance, leading to a reduction in the pulpal levels of extrinsic materials. This response is believed to be due to axon reflex activity; that is, antidromic stimulation of the inferior alveolar nerve causes the simultaneous depolarization of all the nerve terminals providing sensory innervation of the teeth. This release of neuropeptides from the terminals causes both increased pulpal blood flow and extravasation of plasma proteins from the microcirculation (Heyeraas and Berggreen, Berggreen et al).

Alterations in pulpal blood flow may alter the diffusion of substances through dentinal tubules by varying the rate of dentinal fluid outflow. Matthews and Vongsavan speculated that the hydrodynamic stimulation of exposed dentin (for instance, mechanically) triggers the release of neuropeptides from nerve terminals, thereby increasing local blood flow and tissue pressure and stimulating the outward flow of fluid in dentinal tubules (Heyeraas and Berggreen). This outward fluid flow might rinse the tubules free of inward-diffusing noxious substances via "solvent drag." Indeed, Vongsavan and Matthews (2007) demonstrated that Evans blue dye would not diffuse into vital exposed dentin in vivo but would do so in vitro. They speculated that the outward fluid flow in vivo blocked inward diffusion. This notion was tested in vitro by quantitation of the decrease in the inward flux of radioactive iodide when a simulated, outwardly directed, 15-cm$H_2O$ pulpal pressure was applied to dentin disks. This outward fluid movement caused a 50% to 60% reduction in the inward diffusion of radioactive iodide across acid-etched dentin in vitro (Pashley and Matthews). Thus, the stimulation of dentin with open tubules (ie, hypersensitive dentin) should result in higher rates of outward fluid flow and reduce the inward diffusion of bacterial toxins. This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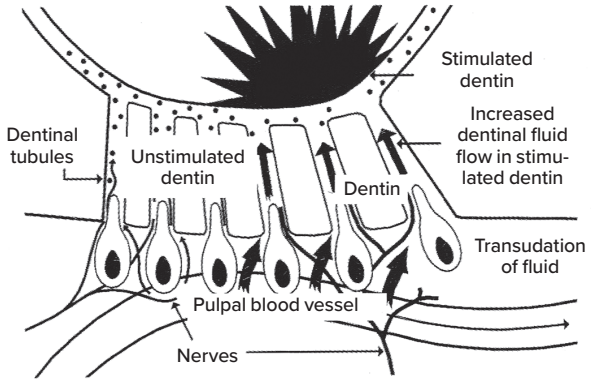
protective effect is diminished by the presence of a smear layer. Under these conditions, the inward flux of noxious substances may increase and permit higher interstitial fluid concentrations to be achieved than would occur if there were more outward fluid movement.

In exposed dentin, outward fluid flow through the tubules is a first line of defense against the inward diffusion of noxious substances. Dentinal fluid contains plasma proteins (eg, albumin, globulins) that bind or agglutinate some materials and may play a protective role (Hahn and Overton). The outward movement of fluid is associated with an outward flux of plasma proteins (Maita et al), including fibrinogen. Pashley et al (1984) reported that dentin removed 61% of fibrinogen as it passed through a 1-mm-thick dentin disk. Presumably, even larger amounts of fibrinogen in vivo would be removed because the tubules contain odontoblast processes at their terminations, which further reduces the sizes of the channels through which this high-molecular-weight molecule must move. This molecule may be responsible for reducing dentin permeability, especially at the pulpal terminations of the tubules, where it may polymerize into fibrin (Izumi et al). Thus, multiple physiologic mechanisms regulate the inward diffusion of substances through dentinal tubules.




▲ **FIG 1-31** Inward diffusion of noxious material *(black dots)* opposed by the outward movement of dentinal fluid in response to the axon reflex–induced release of neuropeptides, which causes vasodilation, transudation, and increased capillary permeability. (Adapted from Pashley 1994.)

## Dynamics of the Dentin-Pulp Complex

To understand the pathobiology of dentin and pulp, it is important to grasp the concept of the dentin-pulp complex. Pulpal cells produce dentin, nerves, and blood vessels. Although dentin and pulp have different structures and compositions, they react to stimuli as a functional unit. The exposure of dentin due to attrition, trauma, or caries produces profound pulpal reactions that tend to reduce dentin permeability and stimulate the formation of reactionary or reparative dentin (Farges et al). These reactions are brought about by changes in fibroblasts, nerves, blood vessels, odontoblasts, leukocytes, and the immune system. Too often, for technical or experimental reasons, the individual components of the dentin-pulp complex are studied independently. However, it is becoming clear that the individual components of the dentin-pulp complex are very interactive and that their activities are interdependent.

Discoveries surrounding the effects of nerves on pulpal blood vessels and vice versa have produced a new appreciation for neuromodulation in response to stimuli applied to dentin (Zhan et al). A plethora of molecules secreted by neurons, including neurotransmitters, neuropeptides, and amino acids, contribute to the induction, sensitization, and maintenance of tooth pain. These molecules are also responsible for the regulation of cells of non-neuronal origin, such as dental pulp stem cells, fibroblasts, odontoblasts, immune cells, and vascular endothelial cells (Liu et al). Dental nerve fibers are also involved in dental hard and soft tissue repair (Widbiller and Schmalz, Olaru et al).

Dentin is a living tissue that reacts to changes in its environment. It is the only innervated hard tissue of the tooth (Luukko and Kettunen). Normally, dentin is covered coronally by enamel and on its root surfaces by cementum, but dentin that is exposed to the oral environment is subjected to extensive chemical, mechanical, and thermal stimuli. The exposed, fluid-filled tubules permit minute fluid shifts across exposed dentin in response to tactile, thermal, osmotic, and evaporative stimuli, which in turn activate mechanosensory receptors on the surfaces of odontoblasts and dental primary afferent neurons (Won et al, Sun et al). These fluid shifts can directly stimulate odontoblasts, pulpal nerves, and subodontoblastic blood vessels by applying large shear forces to their surfaces as the fluid streams through narrow spaces.

The sensory transduction mechanism for painful sensation to hydrodynamic stimuli is more sensitive to provocation by outward fluid flow than inner fluid flow (Charoenlarp et al). Exposure of dentin irritates the pulp and stimulates the release of neuropeptides, such as calcitonin gene–related peptide and substance P, from the pulpal nerves to create a local neurogenic inflammatory condition (Lundy and Linden, Zhan et al). These stimuli also produce changes in pulpal blood vessels, leading to an increased flow of plasma fluid and plasma proteins from the vessels into pulpal tissue spaces and out into the dentinal tubules (Kim et al, Caviedes-Bucheli et al). This extravasation of plasma can also cause increases in local pulpal tissue pressure, which in turn increases the firing of sensitized neurons (Heyeraas and Kvinnsland, Fristad et al). Outward fluid flow may have a protective, flushing function that reduces the inward diffusion of noxious bacterial products in exposed cervical dentin (Fig 1-31).

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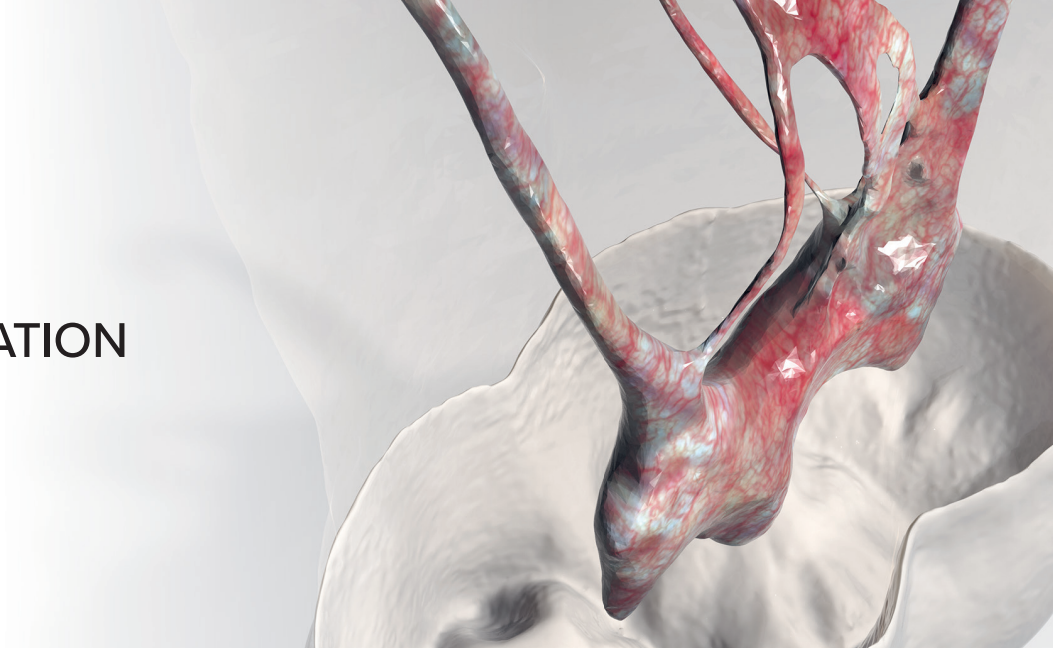

# 2

# DENTIN FORMATION AND REPAIR

Henry F. Duncan
Paul R. Cooper

This chapter provides a detailed discussion of odontoblasts, dentinogenesis, and the traditional classifications of primary, secondary, and tertiary dentin that are used to refer to the various stages of dentin formation throughout life (Kuttler).

## Review of Dentin and Its Many Types

Dentin tissue is approximately 70% hydroxyapatite (Goldberg et al 2011). The dentin matrix is secreted by odontoblasts, with the major organic component (about 90%) being type 1 collagen. Collagen types 3 and 5 have also been detected in dentin (Goldberg et al 2011). Notably, the extracellular matrix (ECM) differs from the pulp and consists of collagen types 1 (56%), 3 (41%), 5 (2%), and 6 (0.5%), along with a population of noncollagenous proteins (NCPs) (Goldberg and Smith). These proteins, including proteoglycans such as chondroitin sulfate and keratan sulfate, represent a small proportion of the matrix (Kawashima and Okiji). The dentin also incorporates a plethora of bioactive components, such as transforming growth factor beta 1 (TGF-β1) and bone morphogenetic protein 2 (BMP-2), which are fossilized during dentin development and contribute to reparative responses in the dentin-pulp complex when released (Smith et al 2016).

There are several types of dentin. Primary orthodentin is tubular dentin that forms before a tooth erupts and continues forming after eruption until root formation is complete. Secondary dentin is also regular orthodentin that forms slowly around the pulp in continuity with the primary dentin for the remainder of the life of the tooth (as long as pulp vitality is maintained). Tertiary dentin is a classification encompassing a range of less regular dentin that may be tubular or atubular in nature and is formed locally in response to tissue irritation or damage, such as caries, trauma, tooth wear, cavity preparation, and restorative dental procedures. Tertiary dentin is generally referred to as either reactionary or reparative dentin, depending on whether the primary odontoblasts survive or are replaced by odontoblast-like cells (Smith et al 1994, Lesot et al). Mantle dentin, the dentin closest to the enamel, is a 15- to 30-μm-thick layer of generally atubular dentin that is initially formed by newly differentiated odontoblasts (Goldberg et al 2011). Mantle, primary, and secondary dentin are the products of secretory odontoblasts that form a complete layer on the formative surface of the tissue at the interface between the pulp and dentin.

## Odontoblasts and the Dentin-Pulp Complex

Odontoblasts located at the periphery of the pulp interface with the dentin to form the anatomically and functionally linked dentin-pulp complex (Pashley).

The dental pulp is located within the center of the tooth and remains active throughout life, providing nourishment to the odontoblasts lining its surface. These odontoblasts have relatively long processes lying within the dentinal tubules that can extend partially or fully to the dentinoenamel junction (DEJ) (Brännström and Garberoglio, Holland). Because of the close interrelationship of the dentin and pulp, the structure is generally referred to as the *dentin-pulp complex* (Pashley). However, this term is not universally accepted, and some authors suggest that the concept of a dentin-pulp complex is an oversimplification (Goldberg and Lasfargues).

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Odontoblasts are tall, columnar cells approximately 50 μm in height with a polarized, basal nucleus and a single cytoplasmic protrusion termed the *odontoblast process* (Couve). This morphology is maintained throughout active dentinogenesis. However, the odontoblast phenotype varies in size and shape in response to changes in secretory activity (Couve, Romagnoli et al, Simon et al 2009, Couve and Schmachtenberg). Because the size of an odontoblast relates directly to its secretory activity, distinct differences can be observed between coronal and radicular cells and can be attributed to their different rates of dentinogenic activity (Kawashima and Okiji). Odontoblast phenotype is defined not only by morphology but also by expression of a characteristic set of molecules leading to matrix deposition that later mineralizes in a regular tubular structure containing the odontoblast process (Butler et al).

The number of odontoblasts also varies with location. Fewer cells are present in the midroot area compared to coronal and apical locations in human premolar teeth; however, the area of intertubular dentin also increases in the coronal portion (Lo Giudice et al). Odontoblast and subodontoblast numbers decrease significantly with age, especially in the root (Murray et al 2002a, Murray et al 2002b). Lower numbers of odontoblasts could potentially reduce the capacity of older teeth to repair tissue and resist injury, but age has not been convincingly demonstrated as a factor for the success of vital pulp treatment (VPT) (Bjørndal et al 2019).

## Embryology of Odontoblasts

Embryologically, odontoblasts differentiate from the dental papilla, which itself originates and differentiates from embryonic ectomesenchymal cells derived from the cranial neural crest at the start of tooth development (Ruch et al, Koussoulakou et al). This shared origin supports the theory of an interconnected existence of the pulp and dentin, which is further supported by laboratory studies that have been unsuccessful in culturing isolated odontoblasts. This lack of success is partly due to the postmitotic state of odontoblasts and also because other pulpal elements are required for their survival and functionality (Klein et al).

Attempts to produce a laboratory odontoblast culture model have resulted in the development of explant cultures in which the entire dentin-pulp complex is used to study odontoblast behavior, either in the form of tooth slice models or in the entire crown (Sloan et al 1998, Pedano et al). These ex vivo systems avoid the use of expensive experimental animal models. Immortalized odontoblast-like cell lines such as MDPC-23 and undifferentiated dental pulp cell lines (eg, OD-21) have also been created to imitate odontoblast-like cell behavior (Hanks et al, Telles et al). Culture of these cells in mineralization-promoting medium containing dexamethasone, β-glycerophosphate, and ascorbic acid results in the development of an odontoblast-like cell phenotype based on morphologic characteristics and molecular expression profiles (Alliot-Licht et al, Duncan et al 2012). Notably, the molecular characteristics of these cells cannot be used to identify them definitively as primary odontoblasts but more simply indicate that they have a mineral-producing phenotype.

## Functions of Odontoblasts

Appreciating the various roles of odontoblasts is critical, both for understanding dentin formation and the response of the dentin-pulp complex to injury and for performing VPT (Bjørndal et al 2019).

The primary function of odontoblasts is to serve as a secretory cell that forms a continuous palisade around the circumference of the pulp tissue in contact with dentin. Beneath the odontoblast palisade is the cell-rich zone of Höhl, which may function to support odontoblast activity, including enabling the interaction between odontoblasts and inner dental pulp fibroblasts (Tziafas). This may include providing a pool of cells that can differentiate into functional odontoblast-like cells after injury (Chang et al). (See the section on reparative dentinogenesis later in this chapter.)

Odontoblasts are postmitotic cells that survive for the life of the tooth and, in addition to their principal function of depositing dentin, are involved in sensory stimulus transmission as well as playing an immunocompetent role in cellular defense (Couve et al). Odontoblasts, along with resident pulp cells such as fibroblasts, immune cells, endothelial cells, and stem cells, express a range of receptors that can detect bacterial components including lipopolysaccharides (LPSs), lipoteichoic acids (LTAs), and bacterial DNA. A well-studied family of receptors is the toll-like receptor (TLR) family, of which 10 members have been identified as being expressed in the pulp (Duncan and Cooper). Regenerative endodontic strategies (Duncan et al 2016a) aim to mimic developmental events in cell-based dentin-pulp regeneration (Chen et al, Yan et al) or whole-tooth engineering strategies (Young et al, Ohazama et al).

The odontoblast layer becomes more crowded as odontoblasts regress toward the center of the pulp following dentin deposition, a behavior that results in the classic S-shaped curve of the coronal dentinal tubules. The morphologic features of active odontoblasts reflect those of other secretory cells, including a basal nucleus with an extensive rough


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endoplasmic reticulum aligned parallel with the length of the cell, a highly developed Golgi complex, and specific secretion granules located in apical areas of the cell and within the odontoblast process (Sasaki and Garant). These features are linked with exocytosis of the secretory granules.

## Dentinogenesis

Dentinogenesis involves two simultaneous processes: organic dentin formation and subsequent mineralization (Linde and Goldberg). In terms of the secretion of organic components, seminal studies using autoradiography to track odontoblast activity and glycoprotein processing in rat incisors have highlighted the secretory pathway used during collagen synthesis. The applied radiolabel was detected first in the rough endoplasmic reticulum and Golgi apparatus before entering the presecretory and secretory granules and finally becoming evident within the predentin at 4 hours (Weinstock et al, Weinstock and Leblond). This same pathway is likely involved in the secretion of other matrix components of dentin, including phosphoproteins and proteoglycans, although the production of some matrix components may be more rapid than for others.

Linde proposed two levels of odontoblast secretion (Linde 1984, Linde 1989). The foremost secretion, described as being at the proximal end of the odontoblast, forms a matrix comprising collagen and proteoglycans, which reaches the advancing mineralization front after approximately 24 hours. The second, located more distally and closer to the mineralization front, is described as the secretion location of various tissue-specific NCP matrix components, including phosphoproteins (eg, dentin matrix acid phosphoprotein 1 [DMP-1]), proteoglycans, and Gla proteins.

The secretions of the organic phase determine the morphology of the dentin, and they are instrumental in the formation of the later mineralization phase (Linde 1989). The NCP components are especially instrumental in inducing hydroxyapatite formation (Linde 1989). This model could potentially explain the formation of peritubular dentin, which possesses a collagen-poor and NCP-rich matrix.

The predentin layer is around 15 to 20 μm thick and is where odontoblast-derived collagen secretions aggregate to form fibrils, which mature and remodel into the meshwork that will form dentin. The width of the predentin layer is a result of the time necessary for this maturation step to be completed (Linde and Goldberg). The intricate process of remodeling occurs as predentin transitions to dentin. This remodeling is particularly related to the proteoglycan components and is orchestrated by the family of tissue proteases called *matrix metalloproteinases* (MMPs) that are expressed by dental pulp cells (DPCs) and odontoblasts (Goldberg et al 2015). MMPs not only degrade proteinaceous ECM components (Verma and Hansch) but are also central to multiple host tissues processes, including differentiation and chemotaxis, because they increase the availability of growth factors (GFs) via cleavage of precursor molecules (Hannas et al). MMPs have been shown to be important for normal and pathologic remodeling processes in various mineralization-associated tissues, including bone (Paiva and Granjeiro), cartilage (Krane and Inada), and dental pulp tissue (Ballal et al, Duncan et al 2022).

Recently, certain MMPs have garnered significant interest within the field of restorative dentistry, with increased amounts of MMP-2 identified in defensive reactionary dentin (Charadram et al) and increased expression of MMP-13 (collagenase-3) observed during dental wound reparative processes (Suri et al, Duncan et al 2022). The importance of MMPs in dentinogenesis is also becoming clearer, with MMP-9 knockout mice reported to exhibit an altered dentin phenotype with reduced dentin volume due to decreased dentin sialophosphoprotein (DSPP) cleavage (Yuan et al) and MMP-13 loss. This resulted in disruption of the predentin layer, tubule/odontoblast irregularity, and a significantly reduced dentin volume in incisors (30%) and molars (13%) (Fig 2-1). This finding was mechanistically related to alterations in Wnt signaling (Duncan et al 2022).

The effects of collagenase on dentin formation have been linked to a disturbance in the ordered secretion of ECM by odontoblasts and its subsequent maturation and degradation (Yuan et al). Critical roles in organizing ECM deposition and facilitating differentiation and GF cleavage from ECM stores have been proposed for collagenases like MMP-13 (Ortega et al, Nkyimbeng et al).

Although dentin has traditionally been regarded as a relatively inert tissue that does not undergo tissue remodeling to the same degree as bone, with an increasing understanding of the role of MMPs and odontoblast function, it is now understood that an elaborate autophagic-lysosomal system exists to ensure turnover and degradation of cellular components to maintain cell functionality (Couve et al). Notably, odontoblasts maintain communication with deeper areas of the dentin matrix through their processes, which lie in the dentinal tubule and traverse the dentin matrix, with lateral branches from the processes connecting with lateral branches of other odontoblasts. The communication between the cell and matrix hints that dentin may not be inert after all.

The mineral component of dentin (like bone) is formed of hydroxyapatite that is closely associated with the collagenous matrix, which orients and supports the mineral crystals (Glimcher). This requires the extracellular delivery of

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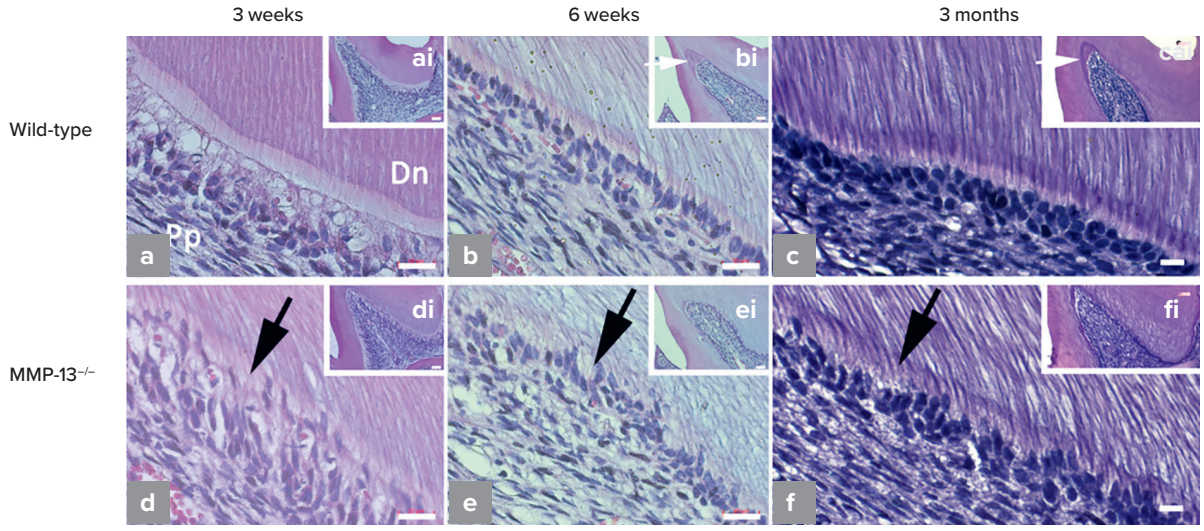


▲ **FIG 2-1** Histologic analysis of maxillary first molars from MMP-13$^{-/-}$ (ie, global MMP-13 knockout) transgenic mice and control wild-type mice. Tissue protease MMP-13 loss critically alters the dentin phenotype in the maxillary first molars of mice. Hematoxylin-eosin (h&e) staining reveals the altered dentin structure, including the disrupted organization of the odontoblast layer, narrowing of the predentin layer, and reduced reactionary dentin formation. (Reprinted with permission from Duncan et al 2022.)

considerable quantities of mineral ions, which will later be deposited as hydroxyapatite crystals in a process that is likely modulated by the subodontoblastic capillary plexus. Odontoblasts have been demonstrated to be capable of transporting calcium and other ions toward the mineralization front (Linde and Lundgren, Linde 1995), where it is postulated that polyanionic NCPs, such as phosphoprotein and proteoglycans, become immobilized on a solid support and induce apatite formation under physiologic conditions. It is generally accepted that heterogeneous nucleation on organic matrix components is responsible for the mineralization of circumpulpal dentin after mantle dentin formation and that the "globular" mineralization front likely arises from the fusion of these hydroxyapatite globules.

## Primary Dentinogenesis

*Primary dentinogenesis* is the widely accepted term for the apposition of orthodentin by odontoblasts during tooth development (Couve et al). The formation of primary dentin begins with the repeated division of the outer cells of the dental papilla. As the cells undergo final mitosis, the daughter cells located subjacent to the basement membrane of the inner enamel epithelium differentiate into preodontoblasts, while the daughter cells located some distance away remain undifferentiated and form the cell-rich zone of Höhl (Goldberg and Smith). Preodontoblasts subsequently elongate and undergo polarization, events that are indicative of terminal differentiation into odontoblasts (Ruch et al).

The dentin matrix is secreted rhythmically, with phases of activity and quiescence—a process that leads to the presence of incremental growth lines perpendicular to the dentinal tubules in mature dentin. These growth lines are produced daily and can be observed on an ultrastructural level in dentin, with the initial and final growth lines at the 5-day interval being approximately 20 µm apart. This pattern of dentinogenesis may be linked to the circadian rhythmic activity of peripheral adrenergic neurons producing variations in the blood flow to the odontoblasts (Larsson and Linde). This explanation, however, does not readily correspond with the patterns of growth lines in the crown and root of the tooth.

### Mantle dentin formation and matrix vesicles

Mantle dentin is formed during the initial stages of dentinogenesis, with mineralization occurring through matrix vesicles (Takano et al). The matrix vesicles are 0.1- to 0.2-µm membrane-bound "sacs" budding from odontoblasts, with the initial crystals formed within the structure adjacent to the large, coarse fibrils of collagen lying perpendicular to the site of the dental basement membrane (Bonucci). No


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vesicles are seen in the later circumpulpal dentin formation stages. Although not understood, vesicles are believed to provide a protected microenvironment for the crystallization and nucleation of enzymes and macromolecules (Bonucci, Linde and Goldberg).

The need for an alternative mechanism of mineralization during mantle dentin formation, which is similar to the vesicles present in bone (Takano et al), may relate to the fact that the odontoblasts are still relatively young and completing their terminal differentiation at this stage. Consequently, they may not exhibit an odontoblast phenotype that is fully capable of the mineralization evident in later circumpulpal dentin formation.

Mantle dentin exists in the outer regions of the crown and is principally an atubular layer, with sparse curved tubules. Similar regions in the root are the Tomes granular layer and the hyaline Hopewell-Smith layer (Goldberg et al 2011).

### Predentin matrix deposition and mineralization

After the formation of mantle dentin, the tightly knit layer of odontoblasts continues to form tubular dentin. Following polarization, the odontoblast processes begin to deposit an unmineralized predentin matrix consisting primarily of type 1 collagen at the inner enamel epithelium (Linde and Goldberg). These collagen fibrils are smaller than those in mantle dentin and the associated noncollagenous organic matrix or ground substance. During the matrix secretion process, the body of the odontoblast moves toward the central pulp while the odontoblast process remains in place and elongates as the organic matrix is deposited around it (Arana-Chavez and Massa).

Mineralization of the predentin matrix to form dentin occurs soon after its deposition. This process is mediated largely by odontoblasts, which produce the matrix vesicles within which hydroxyapatite crystals form in mantle dentin, secrete dentin matrix proteins such as DMP-1 and DSPP, and transport calcium ions and inorganic phosphate to the mineralizing front (Bleicher). The odontoblast-driven mineralization of the surrounding dentin results in the formation of dentinal tubules, with single odontoblast processes remaining embedded within them.

Dentinal tubules increase in density nearing the pulp and confer porosity to the dentin. This permeability has clinical implications for the transport of bacterial byproducts and material constituents and during cavity preparation, which can all readily stimulate pulpal responses (Trowbridge) (see chapter 1). Dentinal tubules are tapered and can vary in diameter from approximately 2.5 mm near the pulp to 0.9 mm near the DEJ.

### Sclerotic dentin

Dentinal tubules can occlude, which lends a translucent appearance to the affected area. This is known as *sclerotic dentin*, which has various origins and is often related to aging. Sclerotic dentin is most common in the apical third of the root, the crown midway between the pulp and outer surfaces of the tooth, and the dentin near the pulp surface. Sclerosis is clinically significant because it reduces the permeability of dentin, which subsequently reduces its sensitivity and the transport of irritants along the dentinal tubules to the pulp. The presence of sclerotic dentin in the roots of adolescent premolars in the absence of any external influence is suggestive of a physiologic response involving the continued secretion of peritubular dentin. However, it might also arise from mineral deposition within the tubule in the absence of peritubular dentin formation, from diffuse calcification within a viable process, or from calcification of both the process and the tubular contents.

### Peritubular and intertubular dentin

As odontoblasts continue to secrete mineral dentin, there are further subclassifications of dentin, including circumpulpal dentin, of which intertubular dentin is the most prominent type. Peritubular dentin, on the other hand, is formed around only the lumens of the dentinal tubules. The relative ratio of intertubular to peritubular dentin varies between species, with peritubular dentin being absent in rodent incisors, high in some animals (eg, horses), and accounting for around 10% to 20% of dentin in human teeth (Goldberg et al 2011). Peritubular dentin is more highly mineralized than the intertubular dentin matrix and contains less collagen, though it is rich in noncollagenous matrix components.

## Secondary Dentinogenesis

Secondary dentinogenesis is a slower process of dentin deposition that occurs after crown and root formation is complete. Secondary dentin, like primary dentin, is produced by primary odontoblasts and is deposited around the entire periphery of the pulp tissue. Importantly, from a clinical perspective, secondary dentin is produced in greater quantities in specific areas, including the floor and roof of the pulp chamber. This leads to a reduced pulp chamber volume in older patients. This dentin deposition, in combination with tertiary dentin, may make carrying out root canal treatment more difficult by making root canals harder to locate and by increasing the likelihood of iatrogenic complications, such as perforation. Although the volume of secondary dentin

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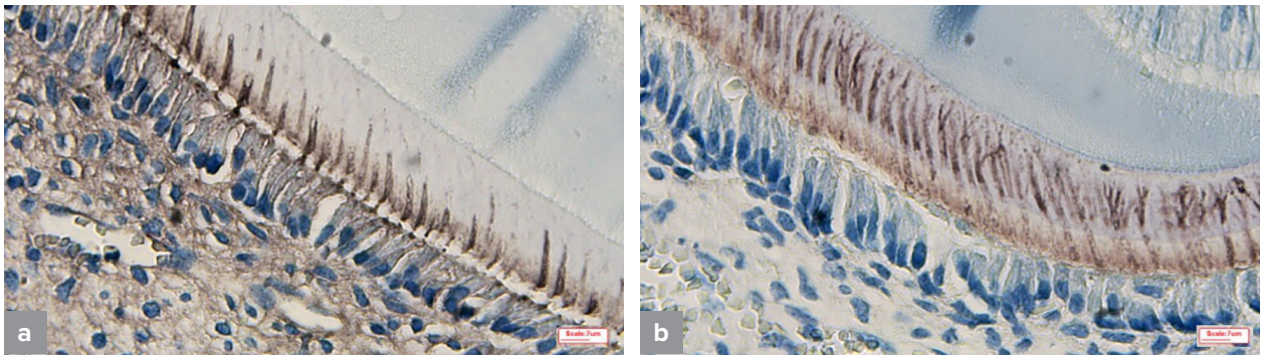


▲ **FIG 2-2** Expression of odontoblast-associated markers in developing rodent molar teeth at 10 days postnatal. *(a)* Immunohistochemistry of the distal cusp of the maxillary first molar highlighting classic nestin staining in the odontoblast layer and predentin. *(b)* DSP expression from a serial section of the same tooth showing a different pattern. Both nestin and DSP are known to be characteristically expressed by secreting odontoblasts. However, they are not unique molecular odontoblast markers. Scale bars = 7 μm; ×40 magnification.

increases physiologically with age, its production has been shown to slow in both the elderly and in women (Solheim). Attempts to use secondary dentin deposition as a means to identify the age of young patients, however, have proved unreliable (Ravi et al).

Because secondary dentin is formed by primary odontoblasts (albeit at a reduced rate), its tubules are continuous with primary dentin. Despite this, it is often confused with tertiary dentin. The chemical composition of primary and secondary dentin is considered identical, with the only difference being the speed of deposition. Odontoblasts secrete primary dentin at a rate of 4 to 20 μm per day but produce secondary dentin at a rate of only 0.4 μm per day (Bleicher). Notably, higher rates of secondary dentin deposition up to 16 μm per day have been reported, most likely due to uncertainty over whether the observed dentin was secondary or tertiary. Although tertiary dentin deposition due to caries or restorative procedures may be obvious, cuspal wear and bruxism will also stimulate the deposition of tertiary dentin in a more subtle way. Furthermore, rodent studies have highlighted dynamic alterations in secondary dentin secretion, with daily deposition rates as high as 16 μm observed at early time periods compared to only 1.25 μm per day occurring after 18 months (Hoffman and Schour).

## Molecular Mechanisms for Odontoblast Differentiation and Deposition

The molecular mechanisms responsible for orchestrating odontoblast differentiation and controlling the rate of dentin deposition are not fully understood. The mechanistic stimuli that slow the deposition of primary dentin to levels associated with secondary dentinogenesis after the completion of crown and root formation could be genetic, epigenetic, or biochemical. To illustrate this, consider a transgenic mouse model. Although initially high levels of DMP-1 were evident in the secretory activity that formed predentin and then transiently high levels in young odontoblasts forming primary dentin, this expression was reduced in mature odontoblasts forming the secondary dentin (Balic and Mina). What controls DMP-1 expression, however, is less clear.

Other studies using transgenic mice have highlighted that transcription factors such as Distal-less 3 (DLX3) play a critical role in odontoblast differentiation, with abnormalities observed in the number and organization of dentinal tubules in mutant DLX3 transgenic mice (Choi et al). Odontoblasts lacking DLX3 exhibited reduced expression of DSPP, decreased dentin matrix secretion, and altered odontoblast polarization (Choi et al, Duverger et al).

Another issue is the lack of odontoblast-specific markers that can be used to definitively identify odontoblast-like cells in culture or their activity in vivo (Duncan et al 2019a). Although dentin sialoprotein (DSP) and nestin are commonly used for this purpose, they are also secreted in the pulp by cells other than odontoblasts and elsewhere throughout the body (Fig 2-2).

In addition to those already mentioned, a wide range of molecules play a role in stimulating odontoblast activity, including BMP-2, BMP-4, BMP-7, TGF-β, fibroblast growth factor 2 (FGF-2), and runt-related transcription factor 2 (Runx2) (Kawashima and Okiji, Smith and Lesot), although it is less clear as to which environmental cues stimulate release processes. GF release can influence ECM secretion, and in turn, ECM molecules modulate GF activity. Transcriptional control of GF expression may also provide a key mode of regulation. Over 500 genes are differentially regulated in odontoblasts involved in primary and secondary dentinogenesis, and these changes


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in the odontoblast transcriptome are associated with activation of the p38 mitogen-activated protein kinase (MAPK) pathway (Simon et al 2009). The upregulation of p38 expression and the activation of p38 protein are observed when odontoblast-like cells are stimulated, such as during tertiary dentinogenesis (Simon et al 2010). Regulation of the MAPK pathway by TGF-β provides some clues as to how odontoblast secretion may be regulated (Zhao et al 2004).

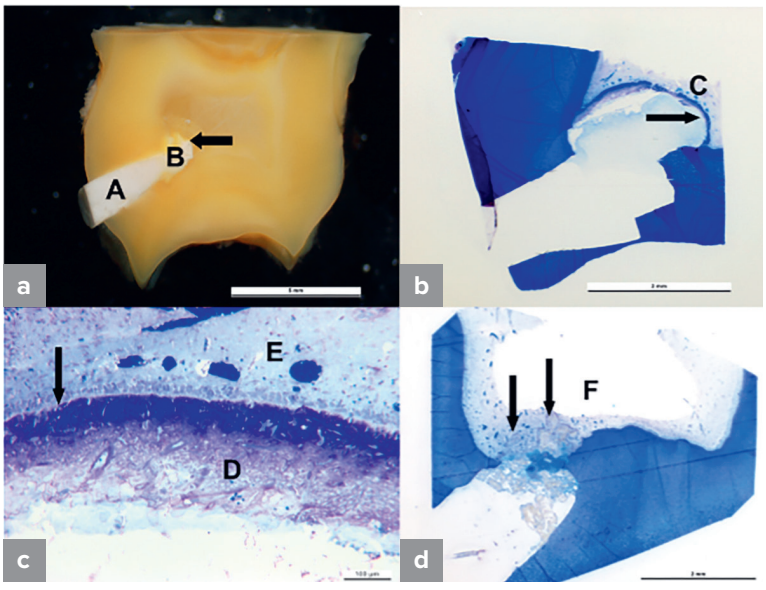
Another pathway that has derived significant attention for its role in odontoblast differentiation is Wnt signaling. Knockout studies in which Wnt ablation was confined to only cells secreting osteocalcin have shown that Wnt regulates pulp and dentin volume, a finding the authors linked to interactions with Runx2 (Lim et al). Recent studies have also identified the role of primary cilia in regulating odontoblastic differentiation through canonical Wnt signaling (Kawata et al), as well as highlighting the critical role of MMP-13 in regulating primary dentinogenesis through Wnt signaling and Axin2 expression (Duncan et al 2022) (see Fig 2-2).

## Tertiary Dentinogenesis

Tertiary dentin is formed in response to dental injury and provides not only a local physical barrier at the point of injury but also contributes to the restoration of tissue integrity via repair and regeneration (Duncan et al 2019a). *Tertiary dentinogenesis* is used to describe a broad range of responses, from the secretion of regular, tubular dentin in continuity with the primary dentin that differs little from primary and secondary dentin to the secretion of a dysplastic, defective, and atubular matrix (Kuttler, Cox et al 1996). Odontoblasts involved in tertiary dentin formation also undertake an immunocompetent role (Couve et al); however, their main function is to secrete mineral alongside the local inflammatory process beneath the damaged area (Lesot et al, Smith 2002) (Fig 2-3).

Various cellular and molecular processes are responsible for the observed range of tertiary dentin formation. Tertiary dentin is classified as either reactionary or reparative, not only to focus attention on the associated cellular processes (Lesot et al, Smith et al 1995) but also to highlight different reactions in response to mild and severe external stimuli. *Reactionary dentin* is tertiary dentin that is secreted by surviving primary odontoblasts in response to a stimulus or irritation (Smith et al 1995). Usually, these stimuli are milder in nature, resulting in an upregulation of the secretory activity of the existing odontoblasts responsible for primary and secondary dentin secretion. *Reparative dentin*, on the other hand, is tertiary dentin that is secreted after the differentiation of a new generation of odontoblast-like cells under the influence of bioactive molecules and is derived in response to a stimulus that has resulted in the death of the original odontoblasts (Lesot et al) (Fig 2-4). Detailed descriptions of reactionary and reparative tertiary processes are provided later in the chapter.

The unpredictable formation of tubular dentin after odontoblast death has led to questions about whether tertiary dentin should be referred to as dentin at all, as well as concerns about the odontoblast-like cell theory. Indeed, the formation of mineral may be attributable to fibroblast cells (Ricucci et al 2014) or fibrocytes (Yoshiba et al) rather than the differentiation of progenitor cells under the influence of bioactive molecules released from the dentin (Smith et al 2012, Smith et al 2016). If fibroblast cells produce the mineral in the reparative response, however, cytodifferentiation would still need to occur because fibroblasts do




▶ **FIG 2-3** Histologic response to pulp capping. *(a)* Macro view of the mesial half of a human maxillary third molar showing remnants of a restorative material and the hydraulic calcium silicate cement mineral trioxide aggregate (MTA) capping material at 1 month. Note the distinct hard tissue bridge *(arrow)* (×8 magnification). *(b)* Photomicrograph of a histologic section of the same specimen with an MTA pulp cap at 1 month. The mineralized barrier *(arrow)* stretches across the entire width of the exposed pulp (×16 magnification). *(c)* Photomicrograph of the same section from *a* and *b* at higher magnification (×85). Cuboidal cells *(arrow)* line the hard tissue barrier. Inflammatory cells are absent in the pulp. *(d)* Photomicrograph of selected serial section of hard-setting calcium hydroxide cement (Dycal, Dentsply Sirona) at 1 month. Engorged blood vessels are prominent and inflammatory cells are present. Note the presence of Dycal particles *(arrows)* in the pulp (×16 magnification). A = restorative material; B = capping material; C = exposed pulp; D = hard tissue barrier; E = pulp; F = pulp. (Adapted with permission from Nair et al.)

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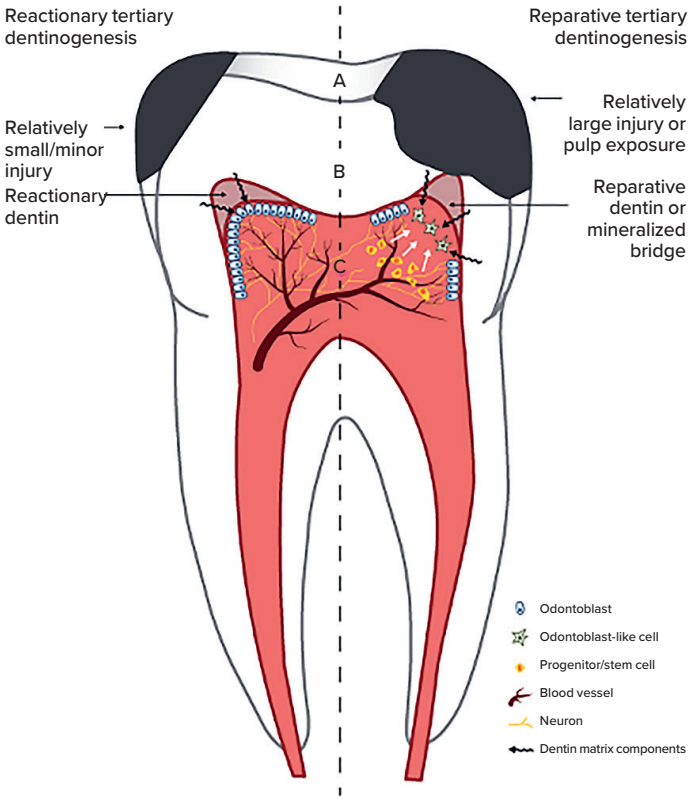


▲ **FIG 2-4** The processes of tertiary dentin formation. Reactionary and reparative dentinogenesis processes differ in the source of the secreting cell. Reactionary dentin is formed by existing primary odontoblasts when a mild stimulus (eg, early carious disease) activates the upregulation of existing odontoblast activity. The odontoblasts recognize bacterial products and released dentin matrix components (DMCs) diffusing through the dentinal tubules, which increases cellular activity. Reparative dentin formation involves a more complex sequence of events in which an intense stimulus (eg, increasing carious involvement of the dentin) causes the death of the primary odontoblasts, which are subsequently replaced following the differentiation of progenitor or stem cells into odontoblast-like cells under the regulation of bioactive molecules (including DMCs). Although the nature of the cellular response is likely dependent upon the pulp environment, the mineralized tissue deposited at the pupal wound site will likely display a spectrum of dysplasia. A = enamel; B = dentin; C = pulp. (Reprinted with permission from Duncan et al 2017.)

not naturally produce mineral in their basal state, although they can be induced to form mineral under certain conditions (Ronchetti et al, Mizoshiri et al, Yamamoto et al 2015, Yamamoto et al 2018).

The histologic assessment of dentinogenic responses is not feasible in clinical situations, and it remains unclear as to how well animal models can replicate the human cellular processes involved in the repair of the dentin-pulp complex. However, considerable data have been compiled on tertiary dentinogenic responses seen in human teeth beneath deep caries lesions (Bjørndal et al 1998, Demant et al), and these studies can provide an invaluable guide to direct clinical treatment planning (Duncan et al).

Both reactionary and reparative tertiary dentinogenesis are defensive processes aimed at regeneration and wound healing. This mineralization response is dependent on the local niche and is accompanied, invariably, by pulpitis, which is helpful if controlled because the inflammation drives the reparative process (Duncan and Cooper). Transient or mild inflammation can promote the tertiary dentinogenic response, but excessive or uncontrolled inflammation can lead to the opposite effect, as evidenced by LPS-induced inflammation in the pulps of ferret teeth, which ameliorated the BMP-7–induced reparative dentinogenesis response (Rutherford and Gu). These bacterially induced inflammatory responses are critical for healing and are described in chapter 7.

From a biologic perspective, the cellular response is not only promoted by the release of GFs and promineralization cues from DPCs (Jeanneau et al) but also orchestrated and regulated by bioactive molecules, including GFs that are "fossilized" in the dentin matrix during dentinogenesis (Cassidy et al, Smith 2003, Grando Mattuella et al). These molecules are subsequently released by caries, irrigants, and dental materials to stimulate the reparative process (Graham et al, Tomson et al 2007, Galler et al 2016, Duncan et al 2017). For didactic purposes, it is easier to consider contributions from the dentin matrix and the DPCs separately. In reality, these elements interact and cross-communicate to facilitate tertiary dentin formation.

## Role of the dentin in tertiary dentinogenesis

Advancing caries progressively demineralizes dentin, which releases dentin matrix components (DMCs) stored within its ECM during tooth development (Dung et al). Select MMPs that are contained within the DMCs, including MMP-2, MMP-8, and MMP-9 (Chaussain et al), further propagate the breakdown of the dentin matrix (Mazzoni et al). This induces the release of other bioactive molecules that diffuse down the dentinal tubules and stimulate tertiary dentin formation and other associated pulpal reparative processes (Finkelman et al, Bègue-Kirn et al 1994, Smith et al 1994). A notable research problem in this area is the dual function of many of the pulpal biomarkers. MMPs specifically are not just destructive in nature but also increase the bioactivity and reparative capacity of DMCs by digesting extracts (Okamoto et al). For example, MMP-13 is proinflammatory (Luchian et al) but is also critical for tooth development and reactionary dentinogenesis (Duncan et al 2022).


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DMCs contain a host of bioactive components, including GFs, chemokines, cytokines, MMPs, and bioactive proteins (Smith et al 2016). These elements modulate a range of cellular processes that are critical for repair, including chemotaxis (Smith et al 2012, Galler et al 2016, Tomson et al 2013), angiogenesis (Roberts-Clark and Smith), mineralization (Tomson et al 2013), stem cell recruitment (Fayazi et al), and neurogenesis (Marquardt et al). GFs are particularly important because they orchestrate and modulate pulpal regeneration along with several members of the TGF superfamily (Cassidy et al, Galler et al 2015) and insulin-like GFs (IGFs) (Finkelman et al, Duncan et al 2017) that are present in DMC extracts derived using ethylenediaminetetraacetic acid (EDTA) and other solutions. A range of other GFs, including angiogenic molecules such as FGF-2, vascular endothelial GFs (VEGFs), placenta GFs (PlGFs) (Roberts-Clark and Smith, Tomson et al 2013), and the neurogenic factors brain-derived neurotrophic factor (BDNF) and growth differentiation factor 15 (GDF-15) (Duncan et al 2017), have also been identified in dentin extracts.

Harnessing the plethora of bioactive molecules available in DMCs for therapeutic purposes has been the focus of considerable recent research activity (Smith et al 2016). It has recently been demonstrated that EDTA (Graham et al, Galler et al 2016), hydraulic calcium silicate cements (Tomson et al 2007), calcium hydroxide (Graham et al), dental resins (Ferracane et al), ultrasonic agitation (Widbiller et al), and epigenetic modifying agents (Duncan et al 2017) can be used to sequester DMCs and augment the regenerative response and stem cell attachment (Sadaghiani et al). EDTA treatment on dentin has been demonstrated to release TGF-β family members from the ECM (Galler et al 2016), whereas sodium hypochlorite treatment causes a deleterious effect on stem cell survival and differentiation ability. This has led to suggestions that the final clinical rinse in cell-homing and VPT procedures should be performed with a 17% EDTA solution (Martin et al). The use of EDTA in these circumstances could enhance the reparative or reactionary dentin response.

### Role of pulp cells in tertiary dentinogenesis

DPCs that are being threatened by an advanced caries lesion respond by expressing a range of genes and proteins to promote defensive cellular processes such as cell migration, proliferation, and differentiation and the recruitment and activation of immune cells (Farges et al). Studies on cell cultures stimulated with LPSs or heat-killed bacteria in mixed DPC populations (Ko et al), selective dental pulp stem cell (DPSC) populations (Li et al), and in vivo (Renard et al) have highlighted alterations in DPC gene and protein expression in an inflammatory environment. Furthermore, cells cultured in mineralizing, angiogenic, and neurogenic culture conditions express a range of extracellular molecules that promote both autocrine and paracrine healing responses (Duncan et al 2013, Gervois et al). Most studies have focused on the reparative role of odontoblasts (Simon et al 2009) or mesenchymal stem cell populations (Frozoni et al). Fibroblasts, however, which are the principal cells of the pulp, are also able to secrete complement fragments and GFs that are important for mineralization and stem cell recruitment (Jeanneau et al). Bone marrow fibrocytes have also been shown to migrate to injured pulp sites to participate in early wound healing processes (Yoshiba et al).

Traditional theory holds that progenitor cells migrate and differentiate to form odontoblast-like cells during reparative dentinogenesis. Several progenitor cell populations may contribute, including DPSCs (Gronthos et al 2002), undifferentiated mesenchymal cells from cell-rich and central pulp perivascular regions (ie, pericytes) (Fitzgerald et al, Machado et al), and possibly stem cells migrating from outside the tooth (Feng et al, Frozoni et al). At present, there is a lack of consensus regarding the progenitor population that is responsible for reparative dentin formation, although surface marker analysis generally confirms that cells of a mesenchymal origin are predominantly responsible for this process (Simon and Smith).

The relative influence of dentin and DPC-derived factors in the formation of reparative dentin is impossible to quantify and is influenced by the short, temporal bioavailability of their expression in cells (Smith et al 2016). What is clear, however, is that both are likely to contribute significantly to the overall repair process in a complementary and possibly synergistic manner.

## Reactionary and Reparative Tertiary Dentinogenesis

Whether a response is reactionary or reparative in nature is not merely a question of semantics. In comparison to reactionary dentinogenesis, reparative dentin formation is a more complex process that typically occurs in response to stronger stimuli, such as extremely deep caries (Bjørndal et al 2019). First, dental pulp stem/progenitor cells are recruited to the site of the injury and multiplied before additional appropriate molecular signaling is performed to induce the differentiation of a new generation of odontoblast-like cells (Lesot et al). All of this must occur before any new dentin matrix formation can take place. Reparative dentinogenesis results in a broad range of dentin that appears to vary in quality, from dentin replacement with evidence of tubule formation to amorphous hard tissue

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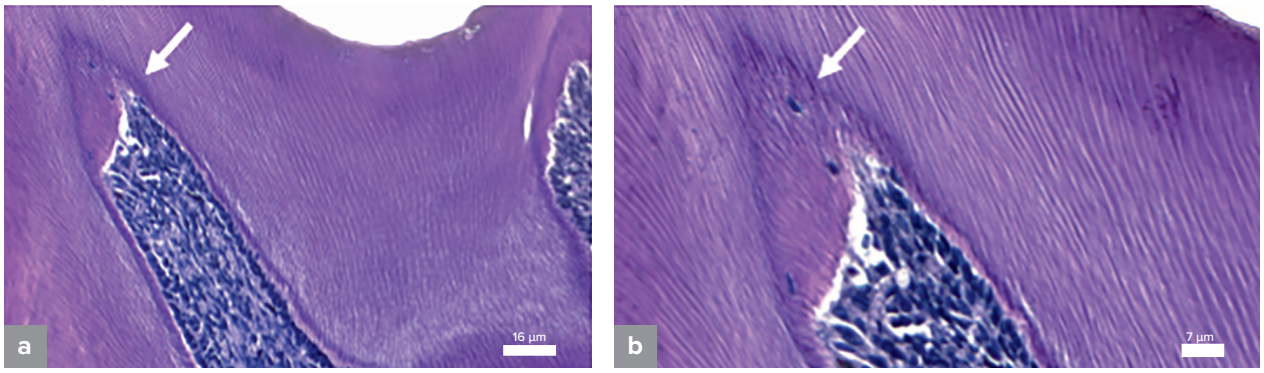


▲ **FIG 2-5** Histologic analysis of maxillary first molars from a 3-month-old mouse. Reactionary tertiary dentin forms a continuous tubular structure in primary dentin. *(a)* H&e staining after cusp wear highlights the reactionary tertiary dentin at the cusp tip. Scale bar = 16 µm; ×20 magnification. *(b)* Higher magnification of the reactionary dentin shows a tubular dentin structure in continuity with the primary dentin. Scale bar = 7 µm; ×63 magnification.

bridges with so-called "tunnel" defects (Cox et al 1996, Nair et al, Ricucci et al 2014, Duncan et al 2019a).

For teaching purposes, the processes of reactionary and reparative dentin formation are generally considered separately. It is likely, however, that in a deep caries lesion both processes will occur simultaneously, particularly at the periphery of the cavity (Smith et al 2016). In advancing caries lesions, reactionary dentinogenesis will probably occur in younger parts of the lesion, whereas in established, more advanced regions of the lesion, reparative dentinogenesis would be expected (Bjørndal et al 1998).

The simple absence or presence of pulpal exposure is insufficient to differentiate reactionary and reparative tertiary dentinogenic processes because local odontoblast death can occur due to traumatic or extremely deep carious injury even in the absence of pulpal exposure. When calculating the likelihood of odontoblast death, the intensity and speed of the injury, as well as the remaining dentin thickness, which serves as a physical barrier and protects against cellular and molecular damage from clinical interventions, are important factors to consider.

## Reactionary tertiary dentinogenesis

It is well established that reactionary dentin is secreted by surviving primary odontoblasts. Identifying primary odontoblasts and determining what mineralization can be attributed to new odontoblast-like cells in histologic studies is less clear. Because histologic analysis provides only a snapshot of the processes involved, the dynamic sequence of events is generally unclear. However, evidence of tubular continuity between primary, physiologic secondary, and tertiary dentin structures has been suggested as evidence of the contribution of primary odontoblasts (Duncan et al 2019a).

### The upregulation process

Reactionary dentin deposition represents an upregulation of the physiologic secretory activity of primary odontoblasts. It is regarded as tertiary dentin because it is stimulated by irritation or injury to the dentin-pulp complex; therefore, matrix deposition is limited to the odontoblasts that maintain tubular continuity with the irritating stimulus (Fig 2-5). The severity and nature of the inciting injury determine the reactionary dentin response, both in terms of volume and in the quality of the dentin deposited. Deeper caries lesions will likely stimulate reactionary dentin formation directly under the cavity (Murray et al 2002a).

### Mechanistic considerations

It was traditionally believed that bacteria, bacterial components, and restorative products that have leached down dentinal tubules stimulate an initial inflammatory response and subsequent repair processes. This thinking, however, has changed. Studies in which DMCs were placed directly in either unexposed cavities to ensure odontoblast survival (Smith et al 1994) or in the exposed pulp after odontoblast death (Okamoto et al) have demonstrated the role of dentin-bound components, including GFs and chemokines, in stimulating tertiary dentinogenesis. Other studies have also provided supporting evidence of the involvement of dentin-bound individual members of the TGF-β family following their application in tooth slice models (Melin et al) and directly in pulp in vivo (Nakashima, Tziafas et al 1998).


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When isolated DMCs were placed in unexposed cavities created in ferret teeth, there was an increase in reactionary dentinogenesis and an upregulation of odontoblast activity (Smith et al 1994). Chromatography of the active matrix components prior to implantation and assay for GFs indicated that this material contained significant amounts of TGF-β1, which is known to influence odontoblast differentiation and secretory behavior. Indeed, TGF-β1, TGF-β2, and TGF-β3 isoforms are known to be expressed by odontoblasts (Sloan et al 2000), and TGF-β1 becomes sequestered within the dentin matrix during formation. After cavity preparation, trauma, or carious attack, these DMCs are released and traverse the dentinal tubules to stimulate odontoblast and pulp cell activity. In addition to TGF-β and other members of the TGF superfamily, other chemokines and cytokines are also involved in this process (Smith et al 2016). Release of these dentin-bound matrix components can be induced with etchants (Galler et al 2015), EDTA (Duncan et al 2017), ultrasonic activation (Widbiller et al), and even dental materials such as calcium hydroxide (Graham et al) and mineral trioxide aggregate (MTA) (Tomson et al 2007), albeit demonstrating different GF release profiles.

Although attention has focused on the effects of TGF-β, other GFs including BMPs (Bessho et al), hepatocyte GF (Tomson et al 2013), IGFs (Finkelman et al), and angiogenic factors (Roberts-Clark and Smith), among others, have all been shown to be contained with the dentin matrix and can stimulate DPC differentiation. It is also important to consider other bioactive molecules as well, including adrenomedullin (Musson et al) and MMPs (Smith et al 2016). Study data support the premise that dentin contains a rich source of mediators that form a tissue-regenerative cocktail when released from damaged dentin.

### Factors influencing the reactionary dentinogenesis process

Tertiary dentinogenic responses beneath caries lesions are easily recognized (Magloire et al), and the activity of a lesion significantly influences the nature of the response. Reactionary responses are often associated with relatively small and slowly progressing lesions (Bjørndal and Darvann, Bjørndal et al 2019). In more active lesions, death of the primary odontoblasts is more likely, and reparative dentinogenesis will occur only if prevailing tissue conditions allow. Even in slowly progressing lesions, the response is likely to be a mixture of reactionary and reparative dentinogenesis (Duncan et al 2019a, Duncan et al 2019b).

The tertiary dentinogenic response is also influenced by various factors associated with cavity preparation and restoration, including the method of cavity preparation, the dimensions of the cavity, the remaining dentin thickness (RDT), etchant application, and the dental materials used for the restoration, as well as the method of their application (Murray et al 2000a). This highlights the need for careful control of cavity preparation conditions in studies involving histomorphometric analysis of pulpal responses. The use of animal models (Simon et al 2008) to assess pulpal responses to restorative factors can allow for more reproducible control of these factors, whereas in vitro organ culture approaches offer opportunities to examine at least some of these factors, potentially in the absence of inflammation and bacteria (Murray et al 2000b). Nevertheless, it remains important to assess human dental responses to these factors in case of variations across species and to attempt to quantify their relative importance.

The complex interplay between the various factors influencing tertiary dentin responses makes it difficult to unravel their involvement in different phases of injury and repair. However, understanding the interplay between inflammation and regeneration may be critical (Duncan and Cooper). One histologic study performed with young human premolar teeth scheduled for extraction investigated reactionary dentin formation after standardized class V cavities were prepared (Murray et al 2000b). Logistic regression highlighted a strong correlation between patient age and the area and width of the cavity floor, with RDT being the most significant factor determining the speed of tertiary dentin deposition, significantly more important than the material used for restoration (Murray et al 2000b). This work was subsequently developed into a larger human study by the same researchers, who highlighted that the maximal reactionary dentin response occurred when RDT was between 0.25 and 0.5 mm. Critically, odontoblast health was maintained at around 85% in cavities > 0.25 mm RCT, and the population of odontoblasts was reduced to 50% in cavities < 0.25 mm (Murray et al 2002a). A later study confirmed that 0.5 mm was the critical RDT to maintain odontoblast health (Murray et al 2000a). Notable from a clinical perspective is the fact that estimating the exact RDT is almost impossible (Fig 2-6).

Although not statistically as important as RDT, the choice of dental restorative material was shown to influence the survival of the underlying odontoblasts, with calcium hydroxide resulting in a more beneficial effect than resin-based composite and glass ionomer (Murray et al 2002b). Other interventions, such as EDTA application, can also positively influence reactionary dentin deposition. One study using rodent teeth found that as EDTA exposure time was increased up to 20 seconds, reactionary dentin secretion also increased (Murray et al 2008). As discussed earlier, this result is likely linked to the ability of EDTA to release DMCs from the dentin

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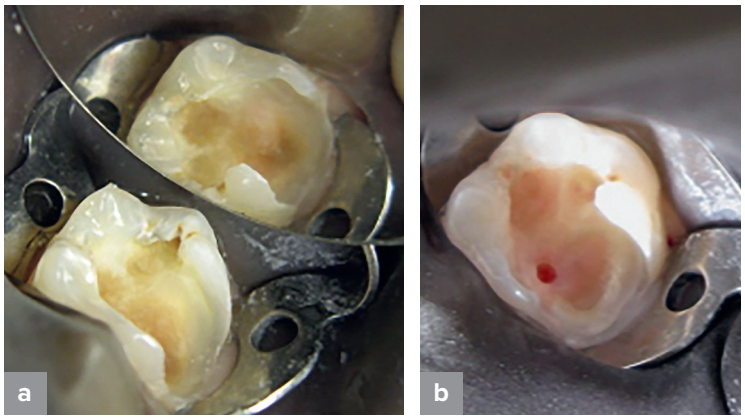


**FIG 2-6** Clinical photograph of a mandibular molar after selective and nonselective caries excavation. *(a)* Selective caries removal. RDT, which is important for odontoblast viability, is difficult to evaluate clinically in a deep cavity. *(b)* Nonselective caries removal exposes pulp and releases dentin-bound GFs and other bioactive molecules into the pulp to stimulate reparative processes.

matrix. The DMCs subsequently diffuse down the tubules and bind to receptors on the odontoblasts (Smith et al 2016). Interestingly, there is a plateau to this effect. Stronger acid etchants, such as phosphoric acid, are less effective than EDTA in solubilizing matrix-bound bioactive components. Mechanistically, the promotion of tertiary dentin relies on multiple factors and can be therapeutically enhanced. However, much remains to be understood, and the most effective way to harness endogenous matrix-bound pools of GFs and other bioactive molecules in the tooth to promote reactionary dentinogenesis remains to be discovered.

## Reparative dentinogenesis

Reparative dentinogenesis may be a sequel to reactionary dentinogenesis as the cavity or caries lesion becomes deeper, it may occur simultaneous with reactionary dentinogenesis before/after exposure, or it may occur independent of reactionary dentinogenesis if the injury is sufficiently intense (eg, aggressive untreated caries lesion or traumatic pulp exposure). As long as the irritation is removed or controlled by therapeutic intervention, reparative dentinogenesis will always take place at sites of pulpal exposure because of the loss of odontoblasts and the need for hard tissue bridge formation. Otherwise, irreversible pulp damage and necrosis will ensue (Mjör and Tronstad 1972, Mjör and Tronstad 1974). As discussed previously, the process of reparative dentinogenesis is biologically more complex than reactionary dentinogenesis in that pulp progenitor cells must be recruited and induced to cytodifferentiate into odontoblast-like cells before secreting the reparative dentin matrix.

The reparative dentin matrix can present with a broad spectrum of appearances, ranging from a regular, tubular matrix to a very dysplastic, atubular matrix, with cellular inclusions sometimes present (Fig 2-7). This extensive morphologic heterogeneity may be attributable to the cellular niche and the presence or absence of caries (Ricucci et al 2020), the type of capping material used (Nair et al), and the technical skill of the operator (Bjørndal et al 2019). Generally, these widely ranging responses are all categorized as reparative dentinogenesis, even in the absence of visible tubular structure. The heterogeneity of responses may reflect a process resembling physiologic dentinogenesis or instead represent nonspecific matrix secretion. Nonspecific secretion can be performed by cells with few phenotypic characteristics of odontoblast-like cells and may represent part of a more generalized wound healing response.

Differences in tubularity in reparative dentin matrices have consequences for the permeability of the matrix and thus affect its ability to protect the pulp from bacteria and restorative materials (see Figs 2-3 and 2-7). Maintenance or re-creation of the tubular physiologic structure of dentin is considered the goal in dentin-pulp regeneration. It is worth considering, however, whether it is truly beneficial. When the pulp must be protected from the effects of bacterial microleakage and components of deep restorative materials, the presence of a tubular, reparative matrix may increase permeability and prove counterproductive. Consequently, a nonporous repair may provide a


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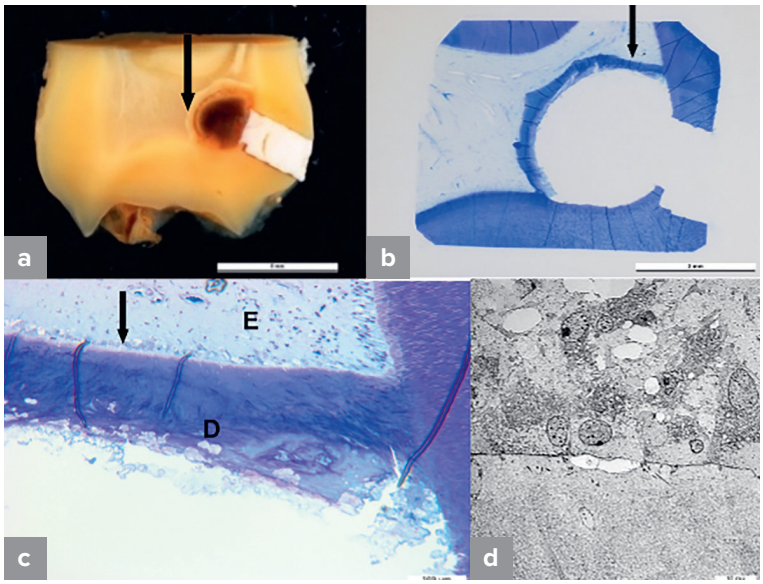
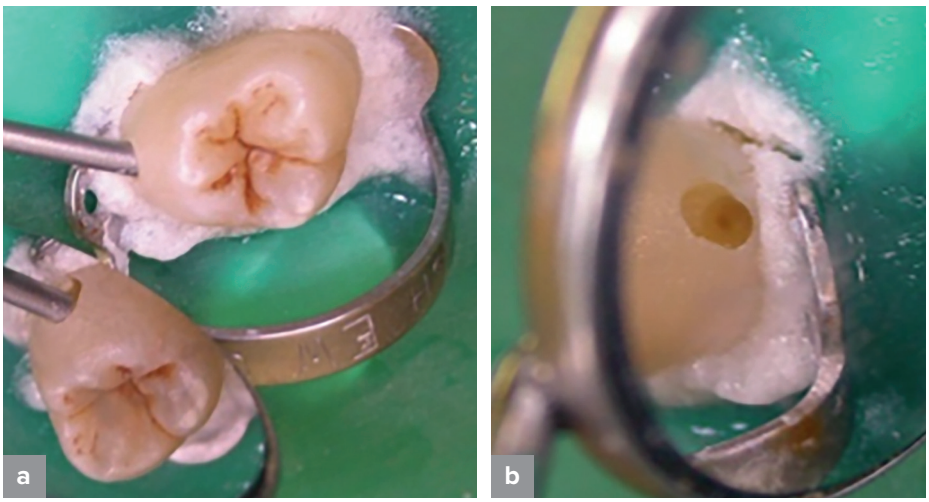



▶ **FIG 2-7** Pulpal response to MTA capping after 3 months. *(a)* Buccopalatal photomicrographic view of a maxillary left third molar showing the cavity preparation and a distinct hard tissue bridge (×6 magnification). *(b)* The mineralized hard tissue barrier *(arrow)* stretches the full length of the exposed pulp free of vascular engorgement (×8 magnification). *(c)* The area under the arrow in *b* magnified (×23 magnification). Note the cuboidal pulpal cells *(arrow)* lining the bridge, early tubule formation, and the absence of pulpal inflammation. *(d)* Correlative composite low-magnification transmission electron micrograph of the bridge and subjacent pulp (×450 magnification). The bridge-pulp interface is magnified, and the bridge is lined with cuboidal cells on the pulpal side. (Adapted with permission from Nair et al.)

superior pulpal defense. On the contrary, the presence of tubular dentin may represent an organized process and the deposition of high-quality mineral rather than amorphous tissue with cellular inclusions that might not form an adequate barrier (Nair et al).

Notably, experimental pulp capping studies investigating reparative dentin responses employ unrestored teeth and relatively short recall periods (Fransson et al) (Fig 2-8). Clinically, however, both traumatic and carious pulpal injuries are affected by infectious, inflammatory, and operator events, and as a result, the interplay between pulp injury, infection, pulpitis, and altered host defense responses over several months are critical to the final outcome (Duncan 2022) (Fig 2-9). A focus of multiple experimental pulp capping studies has been the type of capping material and its role (Aeinehchi et al, Nair et al, Fransson et al) rather than other variables, such as disinfection (Ballal et al), bacterial leakage (Cox et al 1985), and technical aspects of the procedure itself. Indeed, most studies investigating the comparative quality of pulp capping materials do not comment on the technical aspects of the procedure or verify the histologic absence




▶ **FIG 2-8** Experimental pulp capping to investigate reparative dentinogenesis in human teeth. *(a)* Sound, unrestored third molar tooth scheduled for extraction has pulp exposure in a standardized manner in a Class V cavity. *(b)* Although the pulp exposure is standardized, clinical relevance is limited due to the absence of infection and caries in the model.

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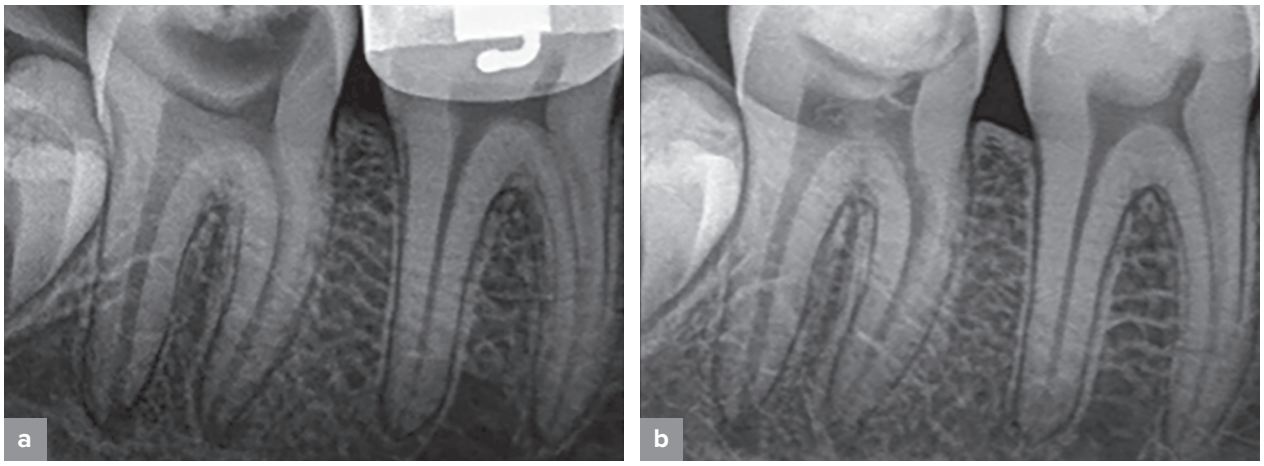


▲ **FIG 2-9** Hard tissue formation visible after a partial pulpotomy at 6 months. *(a)* A deep caries lesion in the mandibular right second molar caused the patient discomfort. *(b)* A hard tissue bridge is evident 6 months after the performance of a partial pulpotomy using hydraulic calcium silicate cement (Biodentine, Septodont). Pulp sensitivity testing gave a positive response. (Adapted with permission from Careddu and Duncan.)

of microorganisms associated with failed or poor healing outcomes (Dammaschke et al).

The process of reparative dentinogenesis is generally preceded by secretion of an atubular fibrodentin matrix that is associated with cuboidal cells at its formative surface (Baume). Later, polarized cells become evident on the fibrous surface and may then produce tubular matrix. It is unclear whether this fibrodentin deposition is specific to the dentin-pulp complex or if it is a nonspecific connective tissue wound healing response. It appears that the fibrodentin plays an important role in the later process of true reparative dentinogenesis by providing a substrate on which signaling molecules for odontoblast-like cell differentiation are immobilized. This could mimic the role of the developmental dental basement membrane for odontoblast differentiation during primary dentinogenesis, where it has been suggested that GFs derived from the inner dental epithelium become temporospatially immobilized to signal preodontoblasts to undergo terminal differentiation (Ruch et al).

Primary and tertiary dentinogenesis are considered comparable events because they share similar cellular and molecular processes (Smith and Lesot, Duncan et al 2022). Indeed, progenitor cell recruitment, the induction of differentiation, and increased secretory activity are common to both, although the environmental niche during repair may lead to diverse secretions. Notably, of course, in reactionary tertiary dentinogenesis there is no need for progenitor cell recruitment or the induction of progenitor cell differentiation.

### Recruitment of progenitor cells

Following VPT, the hard tissue bridge formed over the pulp exposure is generally considered reparative rather than regenerative in nature (Simon et al 2014). However, there remains a lack of consensus on the exact composition of the hard tissue and the nature of the secretory cell that is responsible for its formation (Ricucci et al 2014, Duncan et al 2019a). It has traditionally been theorized that the cytodifferentiation of progenitor cells occurs under the influence of dentin-bound bioactive molecules (including GFs) that are released from the dentin matrix. Consequently, progenitor cells must either be recruited from the pulp core or perivascular areas or migrate into the tooth and undergo subsequent differentiation into odontoblast-like cells before they begin secreting a dentin-like mineralized tissue beneath the site of the lesion (Lesot et al, Frozoni et al, Smith et al 2016, Duncan et al 2019a). To date, however, the exact derivation of the progenitor cells recruited for odontoblast-like cell differentiation during reparative dentinogenesis remains unclear (Duncan et al 2019a).

Tooth development proceeds in the dentin-pulp complex, and it is evident that the pulp comprises various cell populations. Although fibroblasts are predominant, the characterization of DPSC populations has highlighted their heterogeneous nature (Gronthos et al 2000, Gronthos et al 2002), and they have been reported to generate reparative dentin on the surfaces of human dentin after transplantation in vivo (Batouli et al). Because DPSC populations can reside in a perivascular niche


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(Shi and Gronthos), it can be speculated that they derive from the pulp during development or migrate to the pulp through the vasculature postdevelopmentally or at the time of injury. Cells with dentinogenic potential have also been isolated from exfoliated human primary teeth (SHED cells) and the periodontal ligament, and both represent a readily accessible source of stem cells (Shi et al).

Notably, for odontoblast-like cells to be considered morphologically similar to odontoblasts, they must possess an odontoblast process. This would require the hard tissue repair to possess tubularity—a feature that is often absent in reparative hard tissue bridges examined histologically (Nair et al, Ricucci et al 2014, Dammaschke et al), particularly in repairs under caries lesions (Ricucci et al 2014).

Other authors propose the alternative theory that hard tissue formation is part of a reparative scarring process sustained by mineral-secreting fibroblasts (Ricucci et al 2014). Fibroblasts have been shown to produce mineral in vitro (Yamamoto et al 2015, Yamamoto et al 2018) and can contribute to calcification elsewhere in the body (Ronchetti et al). However, to form a dental hard tissue bridge, they would also have to be induced to undergo cytodifferentiation because they do not naturally secrete mineralized tissue. There is no clear scientific evidence to show that this occurs in the dental pulp, with current research suggesting this being only histologically based (Duncan et al 2019a).

Traditionally, undifferentiated mesenchymal cells in the cell-rich zone of Höhl adjacent to the odontoblastic layer were considered attractive progenitor cell candidates because they share a similar developmental history with primary odontoblasts. During tooth development, preodontoblasts align themselves perpendicular to the dental basement membrane during their final mitotic division, with one daughter cell epigenetically induced down the odontoblast differentiation lineage and the other daughter cell assumed to remain residing in the cell-rich zone of Höhl (Goldberg et al 2011). Several cell types, including perivascular cells, undifferentiated mesenchymal cells, DPSCs, fibroblasts, and fibrocytes, are all potentially implicated in dentin-pulp complex repair processes (Duncan et al 2019a).

Stem cells are pluri- or multipotent cells that are critical for the regeneration of damaged tissues in response to growth and differentiation signals. Several dental stem cell populations have been identified, including DPSCs, which account for approximately 1% to 5% of total permanent pulp cell populations (Gronthos et al 2000). Four other human dental stem cell sources have been reported (Huang et al), including stem cells of the apical papilla (SCAP), SHED, dental follicle progenitor cells (DFPCs), and periodontal ligament stem cells (Miura et al, Seo et al, Sonoyama et al). Epigenetic control, including histone modification and DNA methylation, as well as genetic control, appear central to the modulation of stemness and differentiation in various tissue types (Jamaladdin et al), including dental pulp tissue (Gopinathan et al, Duncan et al 2016b). There is certainly evidence in differentiation potential for Notch signaling, which can enable equivalent precursor cells to adopt different cell potentials (Artavanis-Tsakonas et al). The reappearance of Notch in subodontoblastic cells during reparative responses to injury and in association with vascular structures in apical areas of the tooth root (Mitsiadis et al) may indicate that cells in these areas can contribute to reparative dentinogenesis.

#### Odontoblast-like cell differentiation signaling

Following the recruitment of progenitor cells to the site of injury under the influence of the previously described bioactive molecules, progenitor cells must be induced to differentiate to commence tertiary dentin secretion. While epithelial-mesenchymal interactions are required for primary developmental odontoblast differentiation (Koch), this is not understood to be the case in odontoblast-like cells differentiation due to the absence of the signaling epithelium.

Demineralized dentin matrix has been reported to induce this differentiation process (Tziafas and Kolokuris). This theory is supported by the reported promineralization effect of dentin chips and fragments embedded into the pulp tissue during pulp exposure. DMCs, whether whole (Smith et al 1990, Okamoto et al) or in isolated fractions (Bègue-Kirn et al 1994, Rutherford et al), have not only been shown to provide a promineralization response but can also lead to the formation of regular tubular matrix, at least in ferret teeth (Smith et al 1990). Similar responses have been observed when DMCs are substituted with recombinant TGF-β1, with the odontoblast-like cells demonstrating most of the molecular phenotypic characteristics of physiologic odontoblasts (Bègue-Kirn et al 1992, Bègue-Kirn et al 1994).

It has also been proposed that the induction of tertiary dentinogenesis and odontoblast-like cell differentiation could be under epigenetic control. Epigenetic modulation, including DNA-methylation and histone modification, has been shown to be an important regulator of DSC fate (Gopinathan et al). Indeed, histone acetylation has been identified as an important regulator of bone, periodontal ligament (PDL), and dental pulp mineralization processes, as well as being a target for therapeutic manipulation (Duncan et al 2016b, Huynh et al, Ricarte et al, Cantley et al). The acetylation of DNA-associated histone (and nonhistone) proteins is controlled by histone-deacetylase (HDAC) and histone-acetyl-transferase (HAT) enzymes, which alter

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chromatin architecture in response to cellular needs and regulate transcription (Kouzarides) and subsequent differentiation. It is likely that these epigenetic signals triggering odontoblast differentiation by GFs and other molecules may be recapitulated during repair of the adult dentin-pulp complex.

The direct application of GFs, including IGF2 and FGF, to exposed pulp tissue leads to an increase in pulpal mineralization processes and signals odontoblast cell-like differentiation in dogs (Tziafas et al 1998). Similar results have been reported following application of BMP-2 and -4 (Nakashima). These results provide opportunities for the development of biologically based therapies and next-generation pulp capping materials, including materials doped with GFs and other morphogens (Smith et al 2016). This approach, however, is limited by the cost of GFs, regulatory issues, and the short bioactive half-life of GFs (Kearney et al). As a result, strategies to harness the pool of endogenous GFs present in dentin as part of a VPT approach have been promoted (Smith et al 2016). As mentioned previously, this may involve the use of irrigants such as EDTA, materials like calcium hydroxide and MTA, or ultrasonic activation (Smith et al 2016).

Many of the GFs that signal progenitor cell differentiation may be immobilized within the mineralized dental matrix and require unmasking or exposure before they can participate in cellular signaling processes. This has been highlighted by immunolabeling untreated cut dentin surfaces with gold, which has demonstrated TGF-β presentation, although with the absence of reactivity (Zhao et al 2000). Irrigants and cavity etchants unmask TGF-β in the matrix to varying degrees, which highlights that the biologic potential of dentin may be inadvertently altered during restorative procedures. Furthermore, although attention has focused on GFs, tissue proteases (eg, MMP-9 and -13) may also be responsible for cell differentiation (Duncan et al 2016b, Duncan et al 2022), either independently or in combination with GFs. Therefore, the concept that single GFs act independently is probably a simplification, and a combined or additive effect of biomolecules is more likely to occur in vivo.

Evidently, caries, trauma, cavity preparation, and restoration all affect biologic responses through differential solubilization of various dentin-bound molecules. This in combination with the release of cytokines and GFs from DPCs leads to a complex and exquisite response to injury within the pulp. Dead odontoblasts and the inflammatory cells attracted to site of injury further contribute to the milieu by releasing their intracellular contents.

#### The formation of dentin or hard tissue bridges

If a tooth is appropriately restored after pulp exposure, a hard tissue bridge may be formed to restore integrity to the exposed site. Bridge formation represents a type of reparative dentinogenesis and protects the pulp from further insult (Nyborg). The pulpal niche, the extent of the injury, the condition of the pulp, and the reparative processes that are required may all potentially influence the quality and structure of the new matrix secreted within the bridge. Hence, some researchers have called into question whether this represents dentin at all (Simon et al 2014).

Although healing after exposure is described as the formation of a continuous hard tissue barrier over the exposure and a residual pulp that is free of inflammation (Schröder 1973), treatment outcomes for pulp capping can only be evaluated clinically and radiographically (Woehrlen, Fuks et al, Bjørndal et al 2019), so the exact nature of the tissue response is not fully understood. For over a century, pulp capping has been carried out using calcium hydroxide materials (Glass and Zander, Stanley and Lundy, Tronstad, Pitt Ford and Roberts), with commercial products such as Dycal (Dentsply Sirona) developed for this purpose. Applying calcium hydroxide directly onto the pulp can be successful clinically (Brizuela et al) and does induce the formation of a mineral barrier (Schröder 1972), but the barrier that is generated is often porous and of poor quality (Cox et al 1996, Nair et al) (see Fig 2-3).

Although the exact mechanism of calcium hydroxide–induced tissue repair remains unclear (Sangwan et al), the mineralized osteodentin that is predictably formed is of low quality (Cox et al 1996, Nair et al). As a result, new materials are now being preferred, such as hydraulic calcium silicate cements (Koh et al), which have demonstrated improved results histologically (Aeinehchi et al, Nair et al), as well as in clinical trials comparing them with other materials (Hilton et al). Postoperative tooth discoloration has, however, been attributed to the use of MTA (Parinyaprom et al), and newer materials such as Biodentine (Septodont) have potential to overcome this (Careddu and Duncan), while also favorably inducing mineralization (Laurent et al) and cellular differentiation in vitro (Zanini et al). In addition to the biologic effects of calcium hydroxide and calcium silicates on DPCs, they also have the ability, as highlighted earlier, to induce the release of DMCs (Graham et al, Tomson et al 2007). The release of DMCs by pulp capping materials boosts chemotaxis, angiogenesis (Zhang et al), and the differentiation of progenitor cells into dentinogenic cells (Liu et al). Although these effects are promising from a biologic vantage point, there are currently no therapeutic solutions available that use previously extracted DMCs applied directly to the exposed pulp.

New hard tissue bridge formation is considered to represent successful VPT (Eghbal et al, Asgary and Eghbal, Careddu and Duncan). Although this feature can often be observed radiographically, hard tissue bridge formation is


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not predictably visible and, in particular, can merge with capping materials of similar radiopacity (eg, Biodentine). Recently, Taha et al noted that a hard tissue bridge was radiographically detectable in only 8 out of 146 cases at the 1-year follow-up. As a result, few studies report on the presence of a hard tissue radiographically. In a retrospective analysis of 273 pulpotomies, Kunert et al concluded that the radiographic detection of a "dentin bridge" was significantly associated with success, suggesting that this was due to a protective effect (see Figs 2-7 and 2-9). Others have questioned the porosity of the hard tissue bridge formed under calcium hydroxide (Cox et al 1996) and its value in indicating successful healing (Mjör). Its formation does, however, indicate that at least vital cells were present at the time of the bridge formation.

These findings highlight the need to use restorative materials over the capping material that can provide a long-term bacterial seal. Significant increases in late failure have been reported in studies in which capped teeth were restored with glass-ionomer cements (Tan et al). The quality of the coronal restoration was also significantly associated with long-term outcomes in a separate pulpotomy study (Demarco et al). As improved hydraulic calcium silicate materials are introduced and next-generation capping materials are developed, consideration must be given to not only the bioactivity of the material but also its ability to prevent bacterial leakage in the long term.

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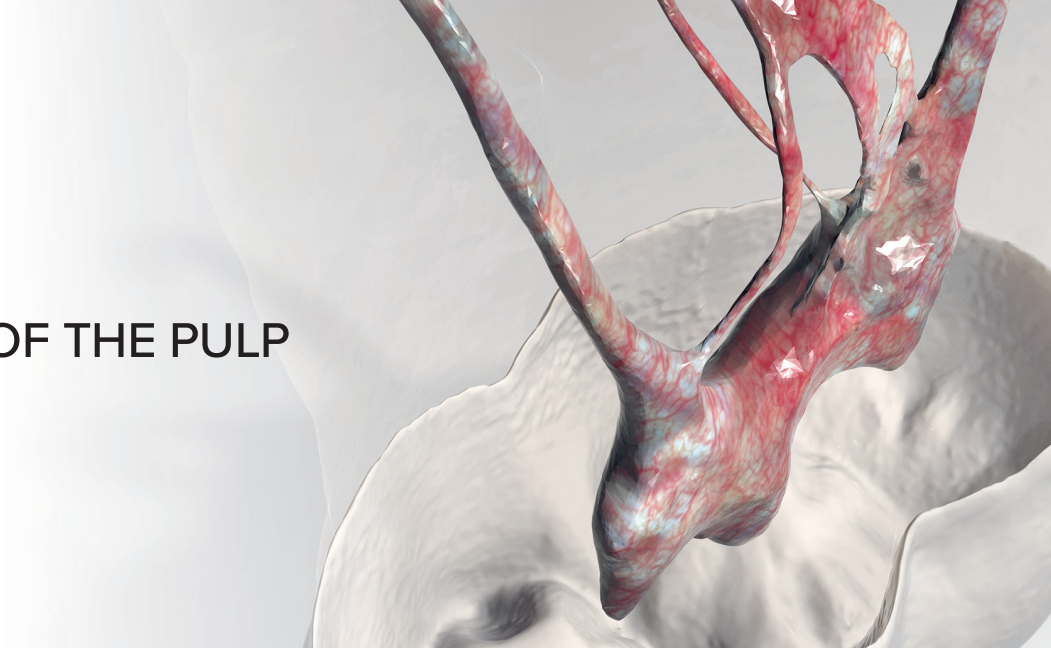

# 3

# CIRCULATION OF THE PULP

Sivakami Rethnam Haug
Bekir Karabucak
Syngcuk Kim

In the 17th century, the father of microscopy, Antonie van Leeuwenhoek, described the pulp as "consisting of such an inconceivable number of blood vessels and other vessels as to surpass all imagination." Charles H. Stowell later described it in the following terms:

> A thoroughly injected pulp, as seen under a binocular, is a most beautiful object. The larger vessels in the center rapidly divide and anastomose, producing a rich network. At the periphery the vessels have become reduced to capillaries, which terminate in fine loops. No pen can either picture or describe this most complicated and yet most beautiful arrangement.

In fact, the dental pulp has the highest blood flow per unit weight of tissue among oral tissues (Kim 1985a, Tönder and Aukland).

The dental pulp serves three principal functions: *(1)* It builds and repairs the calcified tissues in which it is enclosed, *(2)* it responds to irritation by producing patterns of neural activity that give rise to sensations of pain, and *(3)* it maintains the vitality of its cellular constituents by providing sufficient oxygen and nutrients for their metabolic requirements (Van Hassel). The dental pulp is encased in a rigid dentin structure and is considered a low-compliant organ. Alterations in circulatory functions, including blood flow, intravascular and extravascular pressures, blood volume, and capillary permeability, play an important role in initiating pulpal disorders and contributing to pathophysiologic processes. Sound knowledge of pulpal blood flow (PBF) is integral to understanding the pathophysiologic mechanisms of pulpal disorders (Kim 1985a). This chapter elucidates the intricate and complex mechanisms involved in maintaining PBF. The final portion of the chapter describes the clinical implications of pulpal circulatory dynamics.

## Vascular Architecture of the Pulp

The dental pulp is supplied by end arterioles consisting exclusively of small vessels, with arteriolar diameters less than 100 μm and venule diameters less than 200 μm. Because of this, the dental pulp, which lacks true arteries and veins, is said to have a microcirculatory system. Use of the terms *arteriole* instead of artery and *venule* instead of vein is based on structural and functional evidence.

Kramer used the unique India ink suction method in freshly extracted human teeth to reveal the dense vascular network of the pulp at the ultrastructural level. He described the main blood vessels as passing through the apical foramen or foramina and running toward the occlusal aspect of the tooth to give rise to a rich capillary plexus near the periphery of the pulp. The main arterioles have smooth walls and pursue a comparatively direct course toward the coronal part of the pulp. These arterioles, arranged in a mainly occlusal direction, split into many branches that pass toward the periphery to form a subodontoblastic plexus. The venules are much larger than the arterioles and have irregular walls. The largest venules lie near the central part of the pulp. Venous drainage from the subodontoblastic capillary plexus consists of many vessels that mainly run obliquely inward, toward the central part of the pulp, in an apical direction.

The peripheral plexus covers the entire periphery of the coronal part of the pulp and extends for a variable distance into the root canals. Within the root, the peripheral plexus is present only on one aspect of the canal, while on the other aspect there are only the larger vessels running to and from the coronal part of the pulp (Fig 3-1). Although the arterioles and venules enter and exit the pulp mainly via the apical foramen, it is not uncommon, especially in multirooted teeth, to find major vessels in other

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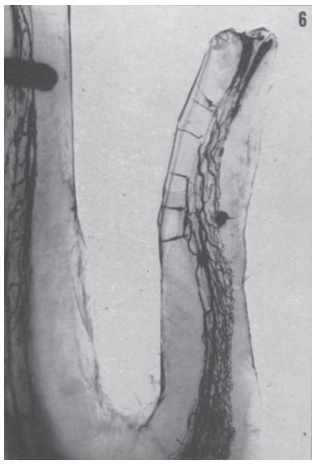
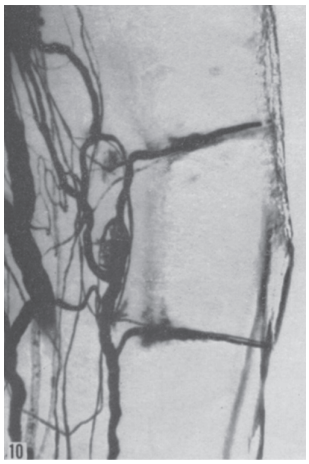
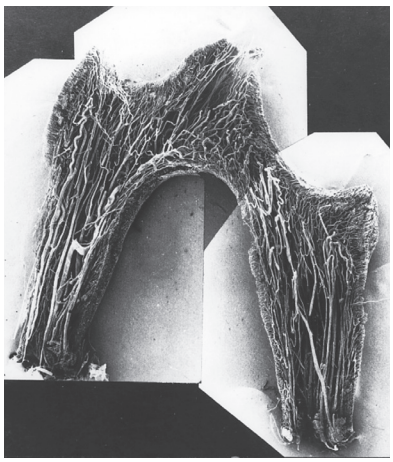


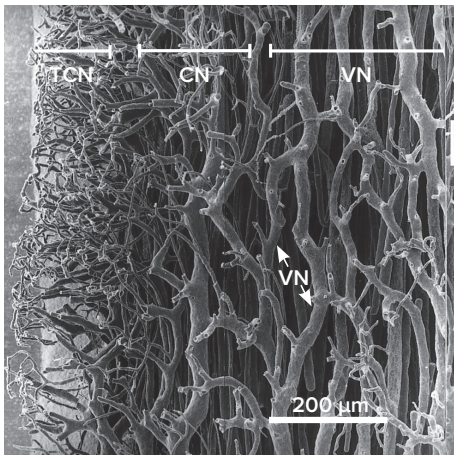
◀ **FIG 3-1** Buccal root of a maxillary first molar. The peripheral capillary plexus in the coronal half of the root canal is present on the outer aspect. Magnification ×14.5. (Reprinted with permission from Kramer.)



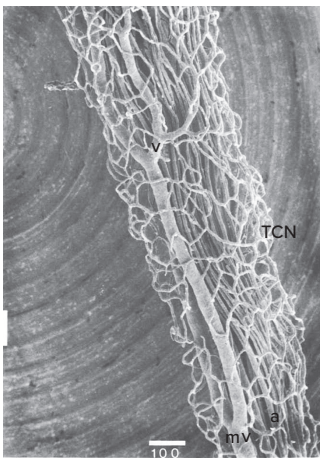
◀ **FIG 3-2** Higher magnification of the root in Fig 3-1. Channels connecting the root canal *(left)* with the PDL *(right)* contain pairs of vessels, one much larger than the other. The smaller vessels resemble arteries, and the larger vessels resemble veins. Magnification ×83. (Reprinted with permission from Kramer.)



▲ **FIG 3-3** Scanning electron micrographs demonstrate the extensive vascular network of the dental pulp in a dog mandibular first molar. The superficial capillary layer has been removed to better illustrate the organizational features of the pulpal circulatory system. (Reprinted with permission from Kishi and Takahashi.)




▲ **FIG 3-4** Scanning electron micrograph of a resin injection cast of dental pulp vasculature after removal of the superficial layer of the vascular network to illustrate the VN, which shows an extensive arborization of capillaries. The TCN is on the left side, the CN is in the middle, and the VN is on the right side. (Reprinted with permission from Kishi and Takahashi.)




▲ **FIG 3-5** Vascular network of the apical portion of a dog mandibular premolar. The network is in a fishnet arrangement. The capillaries of the TCN drain directly into large venules. Scale bar = 100 µm; a = arteriole; v = venule; mv = main venule. (Reprinted with permission from Kishi and Takahashi.)

positions, particularly in the bifurcation or trifurcation region. Smaller vessels running in channels between the root canal and the periodontal ligament (PDL) are relatively common and almost always consist of a pair of one large venule and one small arteriole (Fig 3-2).

The arterial and venous blood flows are joined not only by the capillary plexus but also by direct arteriovenous (AV) anastomoses or shunts, short circuiting the capillary plexus (see section titled "Arteriovenous shunting").

Takahashi created an elegant and detailed 3D representation of the vascular arrangement of the pulpal vessels using microcorrosive resin casts and scanning electron microscopy (Kishi et al, Kishi and Takahashi, Takahashi, Takahashi et al) (Fig 3-3). Scanning electron micrographs show that the superficial layer of the pulp consists of three layers that constitute its basic vascular architecture: *(1)* a dense terminal capillary network (TCN) in the superficial layer of the entire pulpal vasculature, *(2)* the capillary network (CN) in the middle, and *(3)* the venular network (VN) (Fig 3-4).

The TCN is located in the odontoblastic layer and consists of true capillaries. The CN is composed of ascending roots (precapillaries) and descending roots (postcapillaries) running parallel to each other. The VN comprises a fishnet pattern of small venules (Fig 3-5). The main feeding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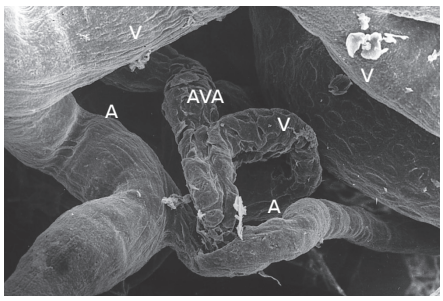
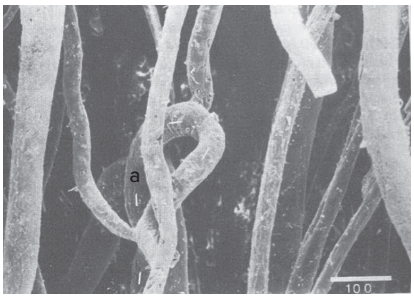
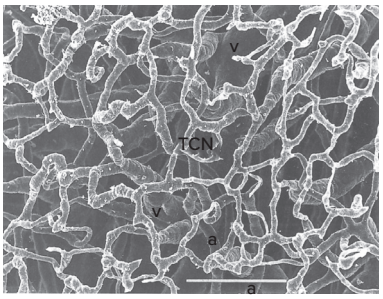


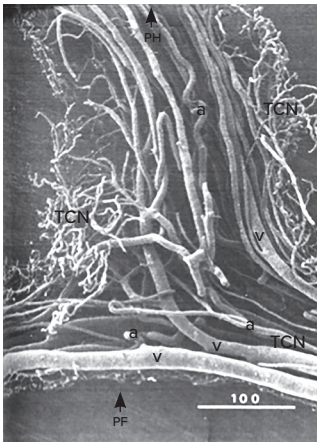

▲ **FIG 3-6** Scanning electron micrograph of a resin cast illustrating the presence of a Y-shaped arteriovenous anastomosis (AVA) between an arteriole (A) and a venule (V) using a sample from a dog tongue (bar = 10 µm). (Reprinted with permission from Kishi and Takahashi.)



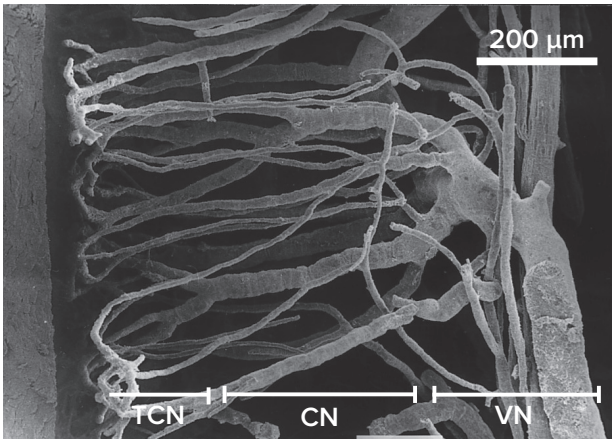

▲ **FIG 3-7** A U-turn loop in the main arteriole is strategically located in the root canal, suggesting that it may play a role in the regulation of blood flow. Scale bar = 100 µm; a = main arteriole. (Reprinted with permission from Takahashi.)




▲ **FIG 3-8** High magnification of a dense CN in the superficial layer of the coronal pulpal vasculature. Scale bar = 100 µm; a = arteriole; v = venule; TCN = terminal capillary network. (Reprinted with permission from Kishi and Takahashi.)




▶ **FIG 3-9** The main arterioles that enter the pulp chamber are separated into two groups; one advances coronally toward the pulp horn, and the other runs between the floor and roof of the pulp chamber. PH = pulp horn; PF = pulp floor; a = arteriole; v = main venule (bar = 100 µm). (Reprinted with permission from Takahashi et al.)




▲ **FIG 3-10** The comb-like hairpin loops of the TCN in the subodontoblastic region. The CN is in the middle, and the VN is on the right side. (Reprinted with permission from Kishi and Takahashi.)

arterioles enter the root canal through the apical foramina and run through the central portion toward the coronal pulp. Some arterioles show AV anastomoses (Takahashi) (Fig 3-6) or U-turn loops (Fig 3-7), wherein numerous arterioles turn in the direction of the dentin and send out many fine branches immediately beneath it. In this area, small vessels form a fine cross-fence TCN (Takahashi et al) (Fig 3-8).

The arterioles that enter the pulp chamber are separated into two groups. One group of arterioles advances coronally toward the pulp horn and branches like a tree toward the dentin to form a dense TCN. The second group of arterioles runs between the floor and roof of the pulp chamber and branches into another dense TCN. These arterioles join arterioles from other root canals (Fig 3-9). The TCN beneath the dentin is almost perpendicular to the main trunk vessels, closely resembling the teeth of a comb in relation to its handle (Fig 3-10).

The microcirculatory bed of the TCN exhibits a continuous progressive hierarchy (arterioles to capillaries to venules), in which the diameter of the venules is larger than the diameter of the corresponding arterioles. A representative capillary diameter is less than 10 µm. The terminal capillaries beneath the odontoblast layer show a

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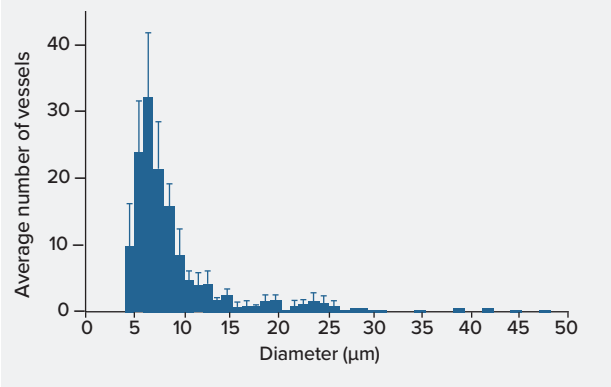
◀ **FIG 3-11** Two loops are mutually connected by an anastomosing branch, the venous-venous anastomosis (vva). v = venule (bar = 100 µm). (Reprinted with permission from Takahashi et al.)




▲ **FIG 3-12** Frequency distribution of the internal diameters of the pulp blood vessels in four cat canine teeth. In each tooth, measurements were made on a section taken 0.5 mm from the pulp horn. The error bars show the standard deviations of the average number of vessels in each bin, except where that number was less than three. (Data from Vongsavan and Matthews 1992c.)

heterogeneous network arrangement. In the pulpal horn area, there is a circular hairpin loop formation, whereas a flat mesh network is observed in the apical portion. The capillaries in the dental pulp have a continuous endothelium, except in the odontoblastic area, where the endothelium may be fenestrated (Corpron et al). Venules occupy most of the area in the TCN beneath the dentin and receive its drainage. These venules coalesce to form joining primary venules, increasing in luminal diameter as they advance toward and through the apical foramina. Primary venules can also form circular loops in the coronal portion of the root canal with short anastomosing branches (venous-venous anastomoses) (Fig 3-11).

Vongsavan and Matthews (1992c) used electron microscopy to estimate that 14.4% of the pulp area they studied was occupied by vessels. In the pulp core, the average value increased to 42.9%, and superficially, near the odontoblast layer, it ranged between 5% and 10%. The capillary density was higher than in most other tissues, ranging between 1,264 and 1,613 capillaries per millimeter. They also found that the largest vessels tended to be near the center of the pulp and that there were relatively few capillaries in this central region (Fig 3-12). Only capillaries with diameters less than 10 µm were present in and near the odontoblast layer (Vongsavan and Matthews 1992c).

A few vascular features in the pulp are unique, including the AV anastomoses, venous-venous anastomoses, and U-turn arteriole loops. There are indications that these features contribute to the regulation of blood flow.

## CIRCULATORY MECHANISMS OF THE PULP

Tissue integrity is dependent on the circulatory transport process, which is influenced by many microcirculatory functional parameters, including blood flow, blood volume, intravascular and extravascular pressures, and capillary permeability (Aukland and Nicolaysen, Rothe and Friedman, Kety).

Intravascular pressure measurements show that a major portion of total pressure dissipation occurs in precapillary microvessels, meaning the arterioles function as resistance vessels. Local and extrinsic stimuli constantly impinge on the walls of the arterioles and exert control over the caliber of these microvessels. Venules function as capacitance vessels, with most of the blood volume of the tissue localized in them. The capillaries are the major exchange vessels. All the nutrients required to sustain cells flow across their surfaces. In most tissues, only a fraction of the capillaries (precapillary sphincters) are perfused.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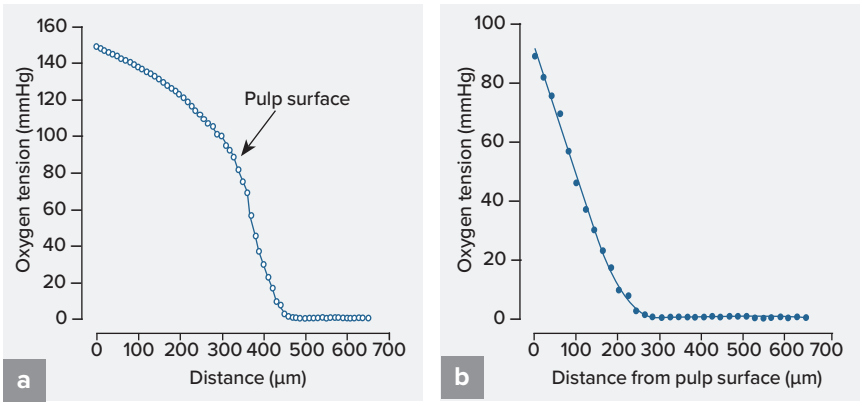


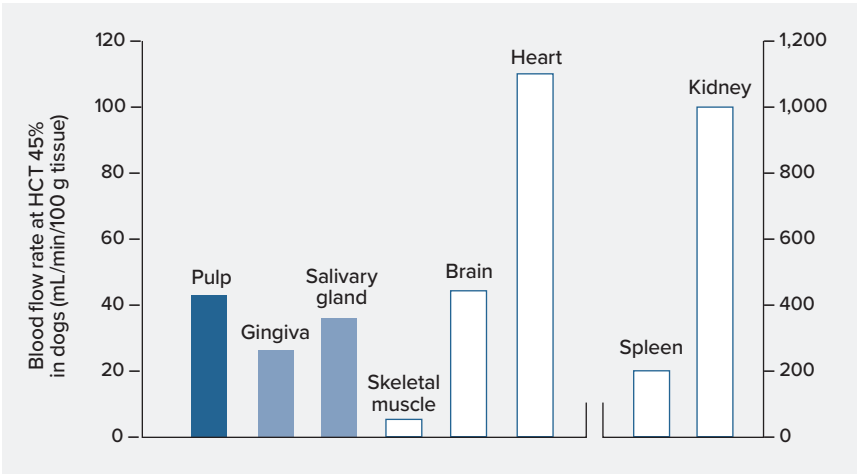

▶ **FIG 3-13** Oxygen profile of a rat incisor pulp. *(a)* Original oxygen profile recorded from the nonperfused pulp. The pulpal oxygen distribution is characterized by a moderate decline in oxygen tension in the saline medium overlying the pulp and a steeper gradient in the interface before a localized oxygen-consuming region (the odontoblastic layer), followed by a plateau in oxygen tension in the middle of the pulp. *(b)* The same oxygen profile as a function of distance from the pulp surface. A fitted curve is superimposed on the data points *(filled diamonds)*. (Data from Yu et al.)

Within rat incisor pulp, it has been shown that pulpal oxygen distribution is heterogeneous and that odontoblasts may be major oxygen consumers. The average oxygen consumption rate of the odontoblasts was found to be about 3.2 $O_2$/min per 100 g of pulp tissue (Yu et al) (Fig 3-13).

## Pulpal Blood Flow

The volume of blood that flows through the vessels per time unit is termed the *blood flow*. Blood flow regulation is essential for the survival and optimal function of the dental pulp tissue. Various methods have been used to measure PBF, including Xe-133 washout and 15-µm radioisotope–labeled microsphere injection methods, the tracer washout method, the $H_2$ gas desaturation technique, intravital microscopy, and laser Doppler flowmetry (Tönder and Aukland, Kim et al 1983, Kim et al 1986, Gazelius et al 1986, Liu et al, Meyer). PBF has been reported to range between 17 mL/min per 100 g tissue weight (Tönder and Aukland) and 129 mL/min per 100 g tissue weight (Kim 1985b). The differences in measured flow values may be due to different methodologies employed, variations in experimental conditions, different stages of tooth development, and heterogeneity of blood flow within the pulp.

The pulp has the greatest blood flow of the oral tissues that have been studied, although it is substantially lower than the blood flow measured for visceral organs such as the kidney, heart, and spleen (Tönder and Aukland, Kim 1985b) (Fig 3-14). Because the pulp is not a metabolically active tissue, one may wonder why the PBF is so high. The high blood flow is most likely a luxury perfusion (ie, the blood flow exceeds what is required for oxygen needs). The kidney and the skin also have luxury perfusion to eliminate various kinds of metabolites and to dissipate heat. It is speculated that the pulp needs high blood flow to prevent the accumulation of various injurious substances, such as bacterial toxins, that can permeate through the dentinal tubules.




▶ **FIG 3-14** Blood flow per 100 g tissue weight for various organs and tissues at 45% hematocrit (HCT) in young dogs. (Data from Kim and Dörscher-Kim 1990.)

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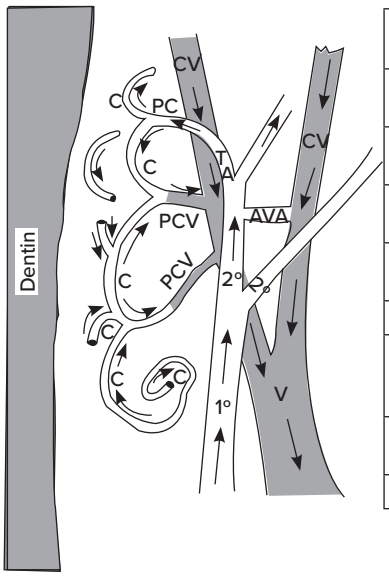


| Vessel type | Diameter (μm) | $V_m$ (mm/sec) | Q ($10^{-4}$ $mm^3$/sec) | Qa/Q |
|---|---|---|---|---|
| 1° feeding arteriole | 35–45 | 1.46 ± 0.11 | 16.68 ± 1.79 | 1.0 |
| 2° feeding arteriole | 24–34 | 1.08 ± 0.09 | 6.31 ± 0.71 | 2.6 |
| Terminal arteriole (TA) | 16–23 | 0.56 ± 0.06 | 1.71 ± 0.21 | 9.8 |
| Precapillary (PC) | 12–15 | 0.48 ± 0.13 | 0.82 ± 0.27 | 20.3 |
| Capillary (C) | > 8 | 0.27 ± 0.03 | 0.16 ± 0.01 | 104.2 |
| Postcap venule (PCV) | 12–23 | 0.20 ± 0.02 | 0.57 ± 0.06 | 29.2 |
| Collecting venule (CV) | 24–50 | 0.37 ± 0.03 | 3.56 ± 0.54 | 4.7 |
| Venule (V) | > 50 | 0.57 ± 0.05 | 16.83 ± 1.75 | 1.0 |

$V_m = \frac{VCL}{1.6}$ (Mean velocity)

$Q = V_m \cdot \frac{\pi D^2}{4}$ (Volumetric flow rate)

◀ **FIG 3-15** *(left)* Tracing from direct microscopic observation of a segment of the pulpal microvascular bed of a rat. *(right)* Tabulation of the distribution of hemodynamic parameters (*D*, $V_m$, and *Q*) obtained from the vessels in the bed. Qa/Q is the ratio of the number of vessels of a given type to the number of primary feeding arterioles. (Data from Kim et al 1984b.)

## Volumetric flow rates

The mean intravascular velocities ($V_m$) of the arterioles, venules, and capillaries have been measured in microcirculatory studies (Kim et al 1984b) (Fig 3-15). The $V_m$ is 1.46 mm/s for primary feeding arterioles (35–45 μm), 1.08 mm/s for secondary feeding arterioles (24–34 μm), 0.58 mm/s for the terminal arterioles (16–23 μm), and 0.27 mm/s for capillaries (> 8 μm). The $V_m$ is 0.2 mm/s for the postcapillary venules (12–23 μm), 0.37 mm/s for the collecting venules (24–50 μm), and 0.57 mm/s for venules > 50 μm. One primary feeding arteriole supplies approximately 104 capillaries, indicating that pulpal vessels have a parallel distribution pattern (Kim and Dörscher-Kim 1990).

The figures showing $V_m$ illustrate that blood flow becomes progressively slower with decreasing vessel size on the arterial side, with the flow being slowest in the capillaries. The relative slowness of capillary flow facilitates the delivery of nutrients and the uptake of waste products from the tissues across the vessel walls. On the venular side, blood flow becomes negligible, yet progressively faster with increasing vessel diameter. Using these velocities, volumetric flow rates can be obtained using the following equation, where *Q* is the volumetric flow rate and *D* is the vessel diameter:

$$Q = V_m \cdot \pi D^2/4$$

In the microcirculatory bed of the pulp, the highest intravascular mean velocity (2.1 mm/s) was recorded in a 42-μm arteriole and the lowest velocity (0.11 mm/s) in an 11-μm postcapillary venule (Kim 1985b, Kim and Dörscher-Kim 1990).

Blood flow in the peripheral layer of the pulp (ie, around the dentin-pulp layer) is four times that found in the central area of the pulp, and the PBF in the coronal area of the pulp is almost twice that of the root area, with the highest flow in the coronal tip (Meyer and Path).

## Blood pressure and flow

The Poiseuille-Hagen equation is used to describe the characteristics of fluid flow through a tube. The fluid moves steadily, and all the particles follow the same line. It demonstrates that as the pressure gradient ($P_1$–$P_2$) along the tube increases, so does blood flow (Q) and also that blood flow is inversely proportional to viscosity (η) and length (L):

$$Q = (P_1 - P_2)\, \pi r^2/8L\eta$$

This equation can be simplified as follows, where *R is the flow resistance*:

$$Q = (P_1 - P_2)/R$$

From these equations, it can be observed that *R* can be expressed as follows:

$$R = 8L\eta/\pi r4$$

This equation indicates that the flow resistance has two components: first, the fluid factor, which is viscosity (η); and second, the tube geometric factors, *L* and *r*.

The Poiseuille-Hagen law was derived from a steady, laminar flow of a homogeneous fluid through a straight, cylindrical tube. In the circulatory systems of animals and humans, however, blood flow is pulsatile; blood vessels are curved, tapered, and branched; vessel walls are distensible; and the blood is not a homogeneous fluid (Kim and


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Dörscher-Kim 1990). Therefore, the Poiseuille-Hagen law cannot be applied quantitatively to blood circulation. Nevertheless, the relationship between pressure gradient ($P_1$–$P_2$) and blood flow (Q) (ie, the resistance [R]) is still a function of vessel geometry and blood viscosity (η). The vessel geometric factor contributing to flow resistance is defined as the vascular hindrance (Z):

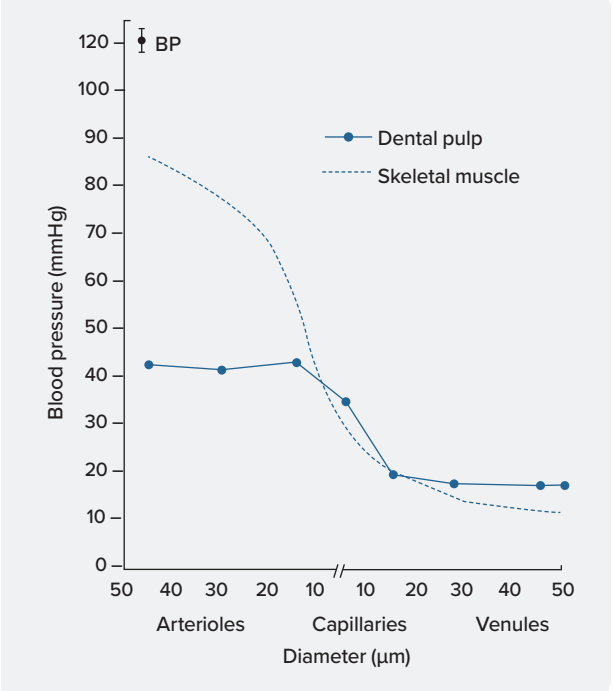
$$R = \eta Z$$

Combining this with our second equation yields the following:

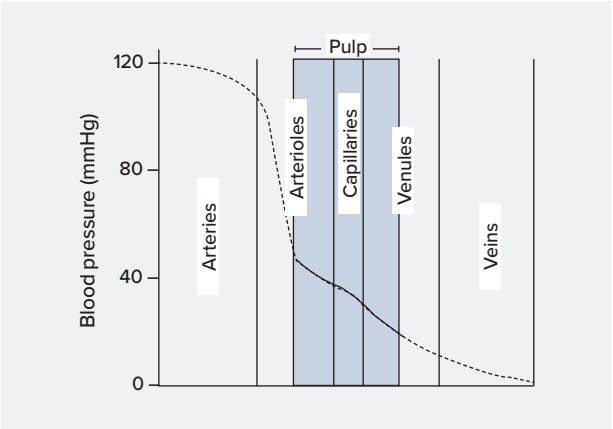
$$Q = (P_1 - P_2)/\eta Z$$

Thus, *blood flow* is directly related to changes in pressure. Moreover, for a given change in pressure, blood flow is inversely related to blood viscosity and vascular hindrance.

Using the micropuncture method, Tönder and Næss (1979) measured pulpal arteriolar and venular pressure. Pulpal arteriolar pressure was found to be approximately 40% of systemic blood pressure, with indirect measurements of about 50 to 60 mmHg. This is low compared to pressure measurements from arterioles of equal size in skeletal muscle and most other tissues (Fig 3-16). The relatively high average blood pressure found in the pulpal venules (19 mmHg, directly measured with micropuncture), however, was not unexpected because thin-walled pulpal venules exist in an environment with relatively high tissue pressure, meaning equal or higher pressure is imperative to prevent their collapse. The pressure in the pulpal venules must also surpass the pressure in the draining mandibular veins, measured to be 16 mmHg (Christiansen et al). In accordance with the low intrapulpal arteriolar and high venular blood pressure measurements, the pressure drop within the pulp itself was found to be low (Fig 3-17), with only about one-fifth of the total AV pressure difference located within the pulp (Tönder and Næss 1979, Tönder 1980).




▲ **FIG 3-16** Blood pressure distribution in relation to vessel diameter in intrapulpal vessels and in skeletal muscle. (Data from Tönder and Næss 1979.)




▲ **FIG 3-17** Falling blood pressure along the extrapulpal *(broken line)* and intrapulpal *(solid line)* blood vessels. (Data from Tönder 1980.)

## Tissue Pressure and PBF

An environment conducive to cellular function is maintained by the continual renewal of pulpal interstitial tissue fluid. This is the medium for the diffusion of substances necessary for the support of cellular metabolism, and all cells, including odontoblasts, must be bathed in tissue fluid.

The capillaries in the dental pulp have a continuous endothelium, except in the odontoblastic area, where the endothelium may be fenestrated (Corpron et al). Water and soluble metabolic substrates are filtered from the plasma across the capillary walls and enter the tissue. The forces promoting filtration are the capillary hydrostatic pressure ($P_C$) and tissue osmotic pressure ($\pi_T$). Plasma osmotic pressure ($\pi_p$) and tissue hydrostatic pressure ($P_T$) oppose the movement of fluid out of the capillaries (Van Hassel, Heyeraas 1985). Marked deviations from normal in any of these pressures, if generalized throughout the pulp, would result in fluid displacements of such magnitude as to be incompatible with a vital pulp (Van Hassel). The net effect of these forces in an average capillary at fluid exchange equilibrium is described by the following equation:

$$P_C - \pi_P = P_T - \pi_T$$

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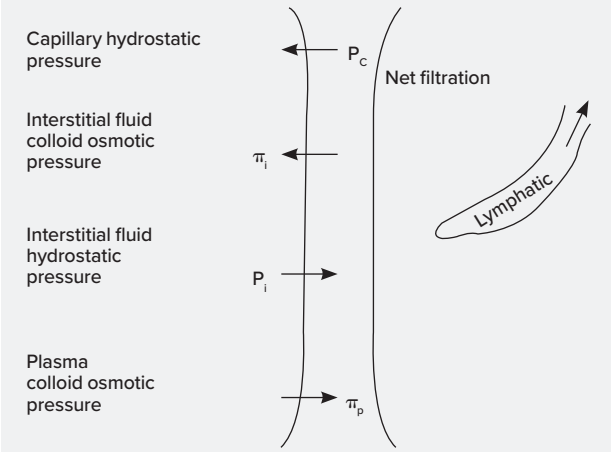
The rate of fluid movement ($J_v$) through the walls of the capillaries is governed by Starling forces (Starling) (Fig 3-18). The filtration and absorptive forces are interdependent. The hydrostatic and protein osmotic pressures operating on each side of a vessel's wall are described in the Starling equation, where *K* is the capillary filtration coefficient (ie, the leakiness of the endothelium), $\sigma$ is the osmotic reflection coefficient, $P_c$ and *IFP* are capillary blood pressure and interstitial fluid pressure respectively, and $COP_p$ and $COP_i$ are the colloid osmotic pressures in plasma and interstitial fluid, respectively:

$$J_v = K[(P_c - IFP) - \sigma(COP_p - COP_i)]$$

These basic forces for transmicrovascular fluid movement are the same in the pulp as in other tissues. Under normal conditions, the microvascular endothelium is somewhat "leaky," and the IFP is slightly negative. On the other hand, the endothelium is quite impermeable to proteins, with a reflection coefficient near 1. Thus, the fluid filtered from the capillaries is low in protein compared to fluid in the vascular space.

The pulp is unique, however, regarding its enclosure between rigid dentin walls, implying low interstitial compliance. In the low-compliant pulp, even modest changes in pulpal interstitial fluid volume (IFV) caused by altered transmicrovascular fluid flow will change the IFP. For example, a raised capillary pressure with enhanced net capillary filtration will be counteracted by a marked increase in IFP, even with a modest increase in pulpal IFV. The low compliance of the pulp tissue is therefore a favorable characteristic, serving as a safety feature and edema-preventing mechanism. Theoretically, a significant increase in IFP may seriously impede PBF, both by compression of venules, thus raising vascular resistance, and secondarily by reducing pulpal perfusion pressure.




▲ **FIG 3-18** The Starling forces governing transcapillary fluid flow. On the arterial side of the capillary, the capillary blood pressure ($P_c$) and the colloid osmotic pressure of the interstitial fluid ($\pi_i$) will tend to move fluid out of the vessel. On the venous side of the capillary, where the blood pressure is lower, the plasma colloid osmotic pressure ($\pi_p$) and the positive tissue pressure ($P_i$) will tend to move fluid back into the vessel.

Several researchers have measured pulpal IFP, or so-called *tissue fluid pressure*, via so-called "fluid equilibration techniques." The most popular fluid equilibration technique for pulpal IFP measurements is the close annulation technique, or tap technique (Van Hassel, Beveridge and Brown, Stenvik et al, Wynn et al). With this method, a low-compliance pressure transducer is connected to the exposed pulp tissue by a saline-filled tube. Another approach for pulpal tissue pressure measurements is the tonometric method, in which an ultraminiature tonometric pressure transducer 1.7 mm in diameter is positioned next to the pulpal interstitium (Christiansen et al). Both the tap and tonometric methods require relatively large areas of pulp exposure. The micropuncture technique was introduced by Heyeraas and uses a pipette with a diameter of 2 to 4 μm (Heyeraas 1985, Heyeraas 1993).

Measurements of pulpal IFP with fluid equilibration techniques have shown great variations in absolute values. Measurements ranging from zero up to about 60 mmHg have been reported in dog pulp using the close cannulation technique (Brown and Yankowitz). The tonometric method showed an average tissue pressure of 17 mmHg, whereas with the servo-nulling micropuncture technique, Heyeraas made a minute exposure in the cat pulp and measured a relatively low IFP in the range of 6 mmHg (Tönder and Kvinnsland).

These conflicting measurements of the absolute magnitude of pulpal IFP are presumably due to the methodologic difficulties of making measurement in tissue of such small size enclosed within dentin walls. The low IFP values between 6 and 8 mmHg seem reasonable for a low-compliant environment such as the dental pulp.

The electrical stimulation of teeth consistently results in an almost simultaneous increase in both IFP and PBF within 6 to 15 seconds, while mean systemic arterial pressure is not affected (Heyeraas et al 1994). Electrical stimulation of the teeth results in an increase in tissue pressure after 30 seconds, followed by an increase in PBF 30 seconds later.

Simultaneous measurements of tissue pressure (micropuncture), blood flow (laser Doppler flowmetry), and systemic arterial pressure before, during, and after electrical pulp stimulation have shown that electrical stimulation consistently causes an early simultaneous increase in pulp tissue pressure and blood flow. Regardless of the length of the stimulation periods, the tissue pressure always reached its maximum before blood flow did. Maximal tissue pressure was recorded after 30 seconds, whereas maximal PBF was measured 61 seconds after stimulation, when the tissue pressure had already started to fall. This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indicates that the instantaneous high rise in tissue pressure limits the blood flow increase to some extent. The fall in tissue pressure with the concomitant maximal flow values strongly suggests increased reabsorption and/or increased lymph flow.

The tissue pressure stayed high significantly longer than blood flow after the 5-minute stimulation periods, indicating increased vascular albumin permeability. However, tissue pressure usually returned to prestimulated levels before blood flow during the longest stimulation period (30 minutes), indicating that interstitial fluid and plasma proteins were removed from the pulp, despite increased vessel permeability due to increased lymph flow, presumably favored by the increased pressure. Repeated long-term electrical stimulation resulted in an increased number of immunocompetent CD43+ cells, and a significantly greater recruitment of immune cells was found in innervated rat first molars compared to denervated teeth (Heyeraas et al 1994, Fristad et al).

The hydrostatic pressure in the interstitial fluid surrounding the pulp cells is the *pulpal tissue fluid pressure*. Compared to most other tissues, the pulpal tissue pressure seems high, 5 to 20 mmHg above atmospheric pressure (Stenvik et al, Tönder and Kvinnsland, Vongsavan and Matthews 1992a).

## Changes in PBF and fluid flow through dentin

There is a continuous, outward flow of fluid through exposed dentin (Vongsavan and Matthews 1992b). This flow is important because it affects the rate at which chemicals diffuse into exposed dentin from the mouth. It also affects both the rate of inward diffusion of drugs and other chemicals applied topically to the floor of a cavity and the bonding of filling materials (Vongsavan and Matthews 1991).

In one study, the average initial rate of outward fluid flow through the dentin was approximately 20.7 pl/s (Vongsavan and Matthews 1992a). Sympathetic stimulation reduced PBF for the period of stimulation and caused a decrease in the rate of fluid flow outward from the dentin. With higher rates of stimulation, reversal of the flow occurred, such that the fluid flow was in an inward direction into the tubules. With sectioning of the inferior alveolar nerve, both dentinal fluid flow and PBF increased and then returned to close to the initial control level after 10 to 15 minutes. Electrical stimulation of the inferior alveolar nerve caused a prolonged increase in PBF and an associated increase in dentinal fluid flow (Vongsavan and Matthews 1992a).

Of particular interest is the evidence that a reduction in PBF, as may occur after the administration of a local anesthetic solution containing a vasoconstrictor, may result in the inward flow of fluid through the tubules, thus facilitating the entry of substances from the exposed dentin surface into the pulp (Vongsavan and Matthews 1992a).

## Pulpal interstitial compliance

Interstitial compliance ($C_i$) is defined as the change in interstitial fluid volume (ΔIFV) divided by the corresponding change in interstitial fluid pressure (ΔIFP):

$$C_i = \Delta IFV / \Delta IFP$$

The compliance determines the change in IFP that results from a given change in interstitial volume. With a low compliance, as in the dental pulp, even minute variations in IFV caused by altered transmicrovascular fluid flow induced by pulpal exposure or the measuring technique will be reflected in the IFP. In most tissues, more fluid is filtered out at the arterial end of the capillary than is absorbed back at the venous end. However, in typical physiologic conditions, IFV in most tissues hardly changes. This is owing to the lymphatics, which drain off the net fluid together with the proteins that leak out of most capillaries.

## The lymphatic system

Any increases in the filtration forces ($P_c$ and $COP_i$) or decreases in absorptive forces ($COP_p$ and IFP) will theoretically result in the progressive accumulation of fluid in the pulp and increase the IFV unless they are balanced by an equal increase in lymph flow. The lymphatic system is a biologic compensatory mechanism for removing filtered molecules and fluids to reestablish homeostasis. Unfortunately, there is no conclusive evidence as to the existence of lymphatic vessels in the dental pulp (Bishop and Malhotra, Marchetti, Marchetti and Poggi, Matthews and Andrew, Dahl and Mjör, Bernick, Kato, Matsumoto et al 1997, Matsumoto et al 2002, Sawa et al, Oehmke et al, Berggreen et al, Virtej et al).

Transport of protein-rich fluid in clefts in the pulp tissue was theorized by Heyeraas, who injected a small volume of radioactive-labeled albumin mixed with fluorescent dextran into the coronal pulp in cats and, at the same time, measured the IFP to control for pressure increases due to the injection (Heyeraas 1989). Injection of a depot of radioactive tracer bound to the plasma protein human albumin into rat pulp chamber resulted in an immediate transport of the tracer within the mandibular canal (Virtej et al). It has been postulated that protein-rich fluid in the interstitial compartment is transported toward the apical part of the pulp and out of the apex, either alone or combined with fluid reabsorption into pulpal microvessels (Virtej et al). Due to the low compliance in the pulp, the

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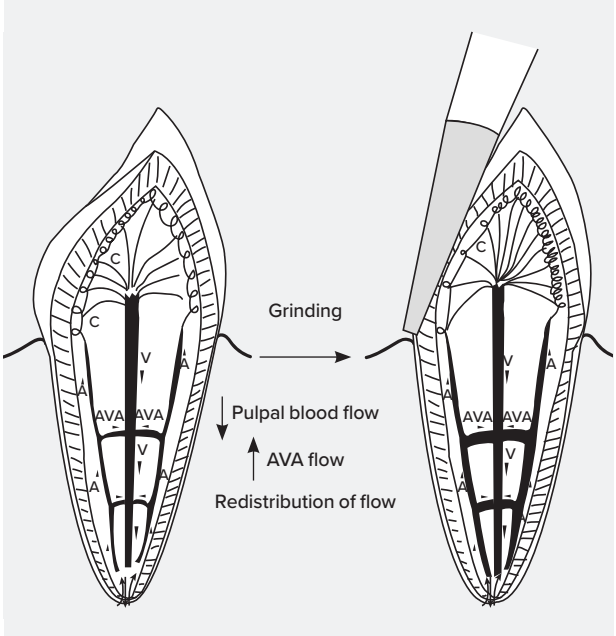


▲ **FIG 3-19** Grinding causes a decrease in PBF and an increase in shunt flow, resulting in a redistribution of flow. The *arrow* represents the direction of flow. The size of the vessels and the degree of flow are proportional. AVA = arteriovenous anastomosis; V = venule; A = arteriole; C = capillaries. (Based on Kim and Dörscher-Kim 1990.)

pulp tissue is under fluid pressure changes due to pulsation in pulpal arteries that may contribute to pulpal fluid movement (Heyeraas and Berggreen). Like the dental pulp, it has been documented that the brain and eye also lack lymphatic vessels. In the brain, interstitial fluid moves through the parenchyma via perivenous drainage pathways to the central nervous system (Smith and Verkman).

Tönder and Næss (1978) used the more advanced servo-nulling micropipette technique to measure not only the pulp tissue pressure but also intravascular pressure. The tissue pressure was measured at an average 6 mmHg, which is substantially lower than previously reported. The pulpal arteriolar pressure through the exposed dentin was approximately 43 mmHg, the capillary pressure approximately 35 mmHg, and the venular pressure approximately 19 mmHg. Figure 3-18 shows the blood pressure profile along the hierarchy of the pulpal microcirculatory network as compared with that of the skeletal muscle.

## Arteriovenous shunting

An interesting characteristic of the dental pulp is arteriovenous shunting. Functional shunting was studied using different size microspheres (Meyer and Path, Path and Meyer). The shunting of 9-µm microspheres, using the 15-µm as nonshunting spheres, for the whole pulp was 33%. The shunting was more prevalent in the apical half (41%) than the coronal half (25%) of the pulp. The functional significance of the shunts can only be speculated to be related to PBF regulation.

It has been shown that AV anastomoses open in response to extensive drilling with intravital microscopy, a response that resembles AV anastomosis opening due to grinding (Fig 3-19). It is possible that the opening of the shunts results from increasing pulpal tissue pressure in response to insult. In this manner, PBF can be maintained in various injurious conditions (Kim and Dörscher-Kim 1990).

# Neural Control of PBF

The dominating mechanism for the regulation of PBF is the nerve component. The pulp is densely innervated with autonomic efferent adrenergic nerves of sympathetic origin and afferent sensory nerve fibers from the trigeminal ganglion.

## Sympathetic nervous system

The postganglionic sympathetic fibers to the dental pulp originate from the cervical sympathetic ganglion, and after joining the trigeminal nerve at its ganglion, most of them follow the course of the sensory nerves to the teeth and adjacent tissues (Matthews and Robinson, Marfurt et al, Kerezoudis et al 1995b). A small but significant proportion of the sympathetic nerves follow another route to the teeth, possibly traveling via the blood vessels (Matthews and Robinson, Haug et al) (Fig 3-20).

Using vital microscopy, Taylor showed that stimulating the transected cervical sympathetic trunk reduced PBF in rat incisors. This finding was later confirmed by Pohto and Scheinin, who also observed that applying an adrenaline solution at the apical foramen of a rat incisor caused a reduction in blood flow in the coronal pulp. Other authors likewise found that stimulation of the cervical sympathetic nerve or infusion of catecholamines caused a reduction in PBF (Taylor, Beer et al, Ogilvie et al, Edwall and Kindlová, Edwall and Scott).

Scott et al used the flying spot method to estimate flow velocity in microvessels and showed that arterioles that were 20–40 µm in diameter have a high flow velocity (mean velocity: 880 µm/s). Sympathetic nerve stimulation caused a 48% reduction in flow velocity (Scott et al). The maximum effect of reduced velocity was observed in large venules (40 µm in diameter). In a typical vessel, the initial velocity of 143 µm/s was reduced by 36% after 4 minutes of stimulation. Cessation of flow often occurs within 20 to 40 seconds. The rate of recovery was rapid, reaching the


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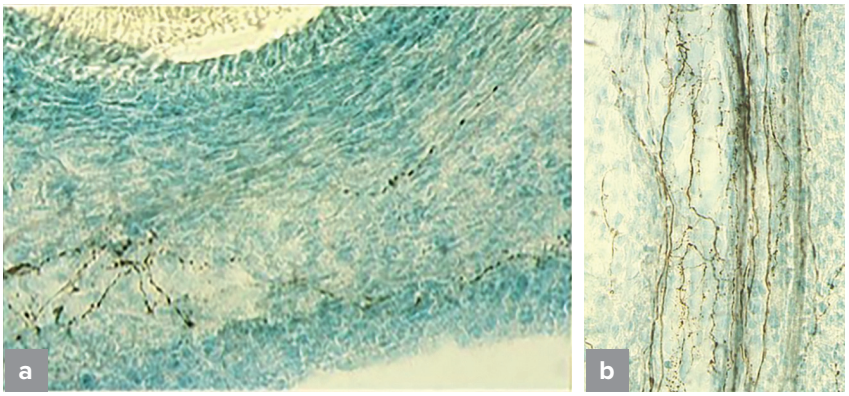
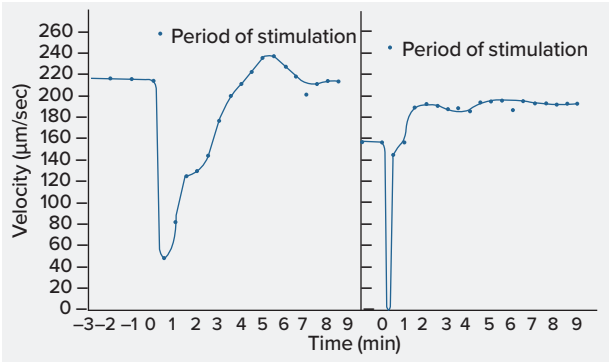


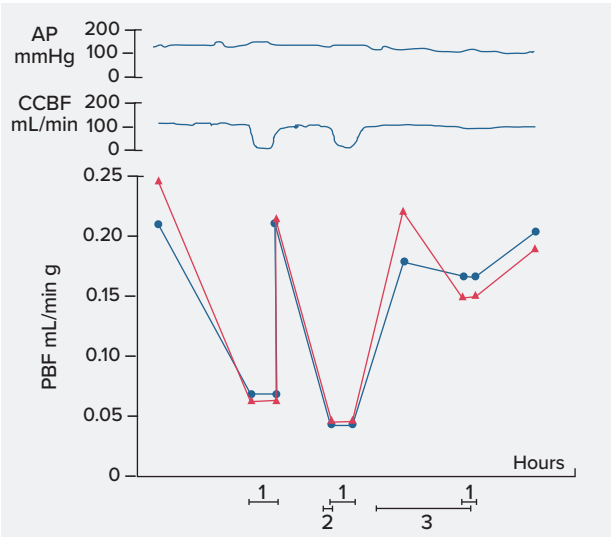

▶ **FIG 3-20** Neuropeptide Y (NPY) immunoreactive nerve fibers around blood vessels in the coronal *(a)* and root *(b)* dental pulp. (Reprinted with permission from Haug et al.)

original velocity within 1.5 minutes of the end of stimulation. This was often followed by a period of supranormal velocity lasting up to 4 minutes. Injecting local anesthetic was found to reduce the resting flow velocity and enhance the effect of sympathetic nerve stimulation so that flow velocity fell to zero within the first 30 seconds (Scott et al) (Fig 3-21). The injection of adrenaline, either alone or with lidocaine, was found to reduce PBF and abolish experimentally induced intradental sensory nerve activity (Olgart and Gazelius 1977), and the reduction of PBF after the injection of adrenaline was always followed by a depression of nerve activity (Edwall and Scott, Olgart and Gazelius 1977). It was therefore concluded that the excitability of sensory neurons in the tooth is strongly modulated by changes in blood flow.

Functional studies have shown that sympathetic vasoconstriction in cat and dog pulp is mediated mainly by vascular receptors (Edwall and Kindlová). Pulpal vessels are equipped with α1-, α2-, and ß-adrenergic receptors. Tönder and Næss (1978) showed that stimulation of pulpal sympathetic fibers reduced PBF by more than 80%, which was prevented by pretreatment with an α-adrenergic receptor antagonist, phenoxybenzamine (Tönder and Næss 1978) (Fig 3-22). The α-antagonist phenoxybenzamine and the α1-antagonist prazosin attenuated the decrease in PBF caused by sympathetic stimulation. Pulpal vessels in the dog, like periodontal vessels in the cat, are equipped with both α1- and α2-adrenoceptors (Edwall and Gazelius, Kim et al 1989, Ibricevic et al). The influence of vasoconstrictor α2-receptors on blood flow in the dog and cat pulp was shown to be smaller than that of α-receptors. The α-adrenoceptors appear to be distributed around both the arterioles and the venules in the rat dental pulp (Kim et al 1989). This means that the remote sympathetic control can selectively regulate the pre- and postcapillary sphincters in the pulp to adjust pressure in the intermediate capillary sections, as required by the tissue.




▲ **FIG 3-21** Changes in flow velocity in a small venule resulting from the electrical stimulation of the sympathetic nerve trunk using 3/s, 6V, and 1 ms. *(a)* Stimulation for 30 seconds just prior to anesthetic application and more than 2 hours after the previous anesthetic application. *(b)* Stimulation for 20 seconds 30 minutes after anesthetic delivery, which immediately followed *a*. (Data from Scott et al.)




▲ **FIG 3-22** Simultaneous recordings of systemic arterial pressure, common carotid blood flow, and PBF in ipsilateral maxillary *(red line)* and mandibular *(blue line)* canines. AP = arterial pressure; CCBF = common carotid blood flow; 1 = cervical sympathetic stimulation (6 Hz, 6 V, 2 ms); 2 = intravenous infusion of atropine (0.3 mg/kg); 3 = intravenous infusion of phenoxybenzamine (50 µg/min). (Data from Tönder and Næss 1978.)

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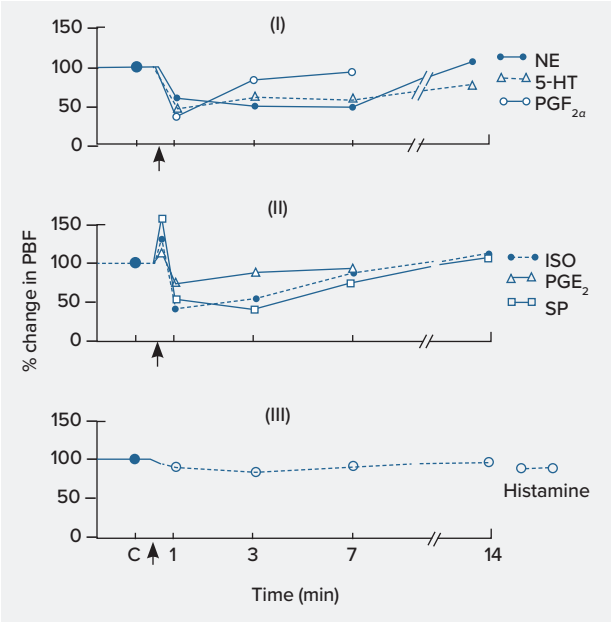
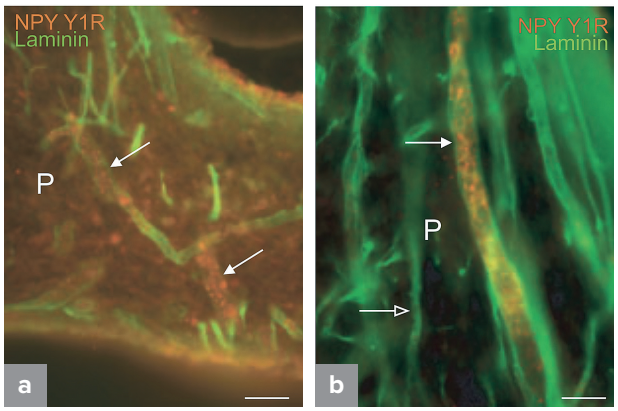



▲ **FIG 3-23** Three types of pulp response to various vasoactive stimuli. (Data from Kim and Dörscher-Kim 1990.)




▲ **FIG 3-24** *(a and b)* Immunofluorescent micrographs of the dental pulp. Perivascular distribution of NPY Y1 receptors *(red)* on blood vessels *(green)*. Scale bar = 50 µm; P = pulp. (Reprinted with permission from Rethnam et al).

The activation of ß-receptors by the intra-arterial injection of isoproterenol caused first a transient increase in arteriolar blood flow and then a reduction; arteriolar dilation was accompanied by venular constriction (Fig 3-23). These flow responses to isoproterenol were blocked by the ß2-antagonist propranolol. Results of microcirculatory studies in dogs and rats have indicated that pulpal hemodynamics are regulated significantly by the sympathetic adrenergic system (Kim et al 1984b, Kim et al 1989).

The paradoxical PBF response following isoproterenol injection in dogs can be explained by several possible mechanisms, including the "stealing of blood flow" by neighboring tissues, as suggested by Tönder (1976); an increase in shunt flow in the pulp microcirculation; a direct constrictive action of isoproterenol on pulpal venules; or a passive compression of pulpal venules due to arteriolar dilation. Of these four explanations, the compression of pulpal venules following arteriolar dilation is the most likely, as pulp exists in a low-compliance environment. This is supported by results found using the microcirculatory technique, which show that there is a transient dilation of feeding arterioles and an initial increase in volumetric flow rate after isoproterenol injection, which occur prior to the onset of flow reduction (Kim et al 1984b).

Tönder and Næss (1978) reported that there is no evidence of tonic activity in the sympathetic supply of the pulp, and cutting the cervical sympathetic trunk had no effect on PBF. At the resting stage, pulpal vessels are not under the tonic influence of sympathetic nerve discharge. The vasoconstriction elicited by cervical sympathetic stimulation was obviated by cutting the inferior alveolar nerve, revealing that the cervical sympathetic adrenergic fibers are contained within it.

Several studies have used histochemical and biochemical approaches to present strong evidence of adrenergic vasomotor innervation in the dental pulp of a variety of species, including humans (Anneroth and Norberg, Pohto and Antila, Kukletová et al, Larsson and Linde, Parker et al, Kerezoudis et al 1992, Luthman et al). Neuropeptide Y (NPY) is another sympathetically derived neurotransmitter that is colocalized in terminals with noradrenaline. NPY and noradrenaline coexist at the sympathetic nerve terminal, and both were found to cause vasoconstriction in the dental pulp (Edwall et al).

Human dental pulp contains NPY in sympathetic fibers, and pulpal blood vessels express the Y1 class of NPY receptors (Luthman et al, Uddman et al, Rethnam et al) (Fig 3-24). Administering NPY into dental pulp reduces PBF via vasoconstriction (Kim et al 1996). This reduction in PBF is similar in magnitude to that produced by electrical stimulation of the sympathetic fibers that innervate the pulp (Kim et al 1996) (Fig 3-25). When stimulated, however, the sympathetic nerve releases NPY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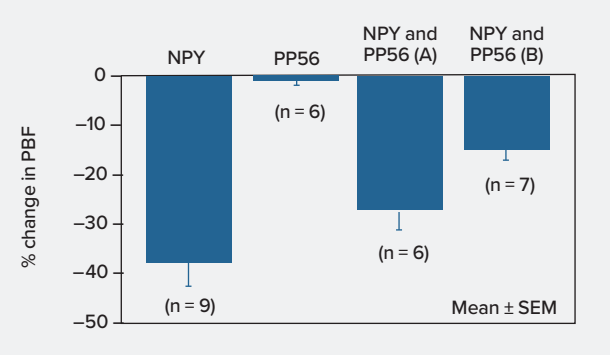
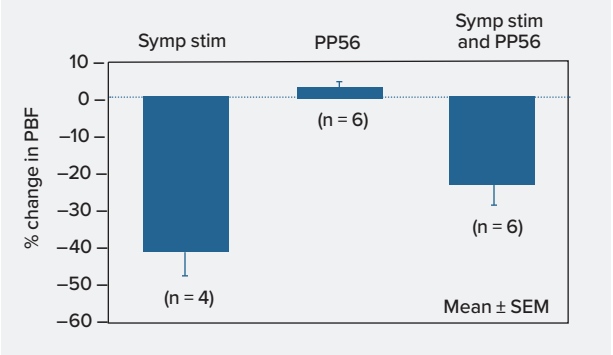



▲ **FIG 3-25** Effects of NPY and its antagonist D-myo-inositol-1,2,6-trisphosphate (PP56) alone and together on PBF in the cat dental pulp. Injection of PP56 (A: 0.3 mg/kg; B: 0.23 mg/kg) significantly attenuated the reduction of PBF caused by NPY (1.3–2.0 µg/kg) in a dose-dependent pattern. (Data from Kim et al 1996.)




▲ **FIG 3-26** Effects of sympathetic nerve stimulation (Symp stim) alone and in the presence of NPY antagonist PP56 on PBF. Injecting PP56 (2.3 mg/kg) intra-arterially significantly attenuated the reduction in PBF caused by sympathetic nerve stimulation ($P < .05$). (Data from Kim et al 1996.)

simultaneously with noradrenaline, and the two work in concert. In one study, electrical stimulation (10 Hz, 4 V, and 1.5 ms for 3.5 minutes) of the sympathetic nerve resulted in a decrease in blood flow of 41.7% 30 seconds after the start of stimulation (Kim et al 1996) (Fig 3-26). Intra-arterial administration of 2.3 mg/kg D-myo-inositol-1,2,6-trisphosphate (PP56, an NPY antagonist), followed by sympathetic nerve stimulation, resulted in a decrease in blood flow of 23.1%. PP56 attenuated the decrease in PBF caused by sympathetic nerve stimulation by 44.4%. Full recovery to control values occurred between 40 and 180 seconds after the termination of stimulation. These findings imply that NPY contributes significantly to sympathetic nerve–induced vasoconstriction (Kim et al 1996).

The sympathetic system is involved in many vital functions, not all related to hemodynamic regulation (Olgart 1996). A major role of the sympathetic system is the maintenance of blood pressure (Olgart 1996). Sympathetic vasoconstriction in cat dental pulp has been shown to have strong effects on the modulation of intradental sensory nerve excitability (Edwall and Scott). Thus, sensory terminals of the A-type readily lose their normal sensitivity during pulpal ischemia (Olgart and Gazelius 1977). For example, a heat stimulus that would excite these nerves under normal conditions was shown to be without effect during a period of sympathetic activation. In animals, a reduction in pain sensation as part of a fight-or-flight reaction may be valuable, but in the human dental pulp, such an effect is not considered useful. Fluctuations in human pulpal pain intensity may be explained by stress-induced sympathetic vasoconstriction. This may be why some patients with pulpal symptoms find that the pain disappears on the way to the dentist. On the other hand, increased activity in the sympathetic nerves and the release of noradrenaline may result in the opposite effect, namely the direct excitation of certain sensory nerve endings. In experimentally induced chronic inflammation in rat cutaneous tissue, a2-adrenoceptor–mediated sympathetic activity was found to excite certain nociceptors (Sato et al). Therefore, a-adrenoceptors present on sensory nerve endings may stimulate pain transmission when activated by noradrenaline and may contribute to fluctuations in the pain symptoms of inflamed pulp (Olgart 1996).

Sympathetic vasoconstriction in the dental pulp and jaws is insignificant for circulatory homeostasis because these tissues represent a relatively small blood volume. The sympathetic vasomotor control of the pulp may have local importance. More recent studies have shown that the sympathetic nerves play an immunomodulatory role in the dental pulp (Haug et al, Haug and Heyeraas 2005, Haug and Heyeraas 2006).

## Sensory nerves

The sensory nerve fibers, which are responsible for sensation in the tooth, have a profound impact on pulpal circulation. The sensory nerves release calcitonin gene–related peptide (CGRP) and substance P along with other neuropeptides from their pulpal nerve endings in response to afferent activation (Gazelius et al 1987, Heyeraas et al 1994, Olgart et al 1977). Most pulpal nerves have been shown to contain CGRP, and approximately three times more fibers are labeled with CGRP than substance P (Heyeraas et al 1993). The activation of sensory nerves in the pulp,

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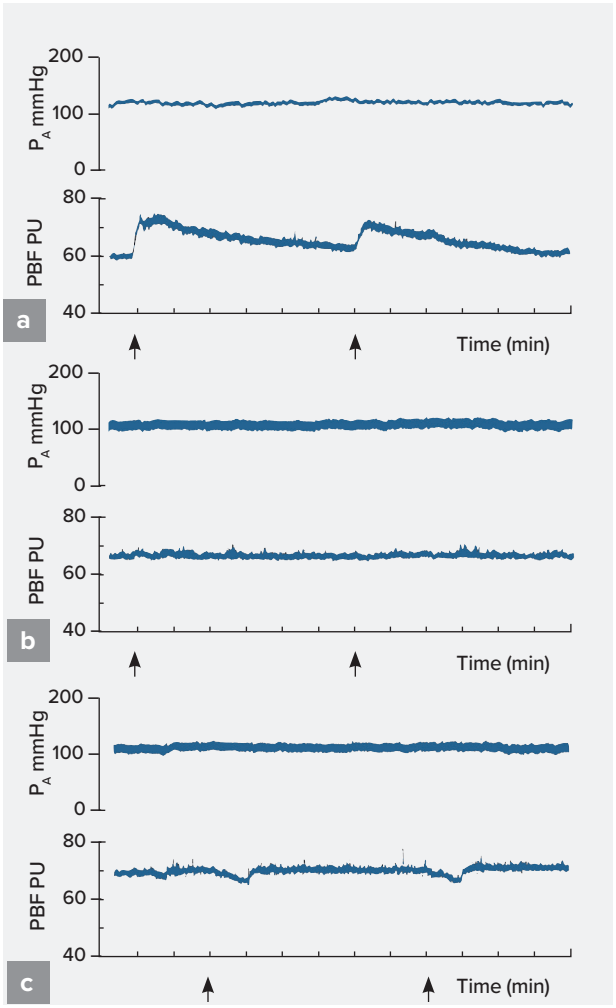
either due to brief antidromic electrical stimulation of the inferior alveolar nerve or direct stimulation of the tooth crown, induces a long-lasting increase in PBF (Tönder and Næss 1978, Gazelius and Olgart, Kerezoudis et al 1993b). Electrical stimulation of the tooth crown results in increased blood flow due to the release of sensory neuropeptides such as CGRP and substance P, and repeated electrical stimulation results in the marked reduction or depletion of CGRP and substance P in the dental pulp (Jacobsen and Heyeraas 1997, Fristad et al). The mechanism of this reaction was shown to involve both substance P and CGRP in rat incisor pulp (Kerezoudis et al 1994a, Kerezoudis et al 1994b). Specific antagonists have been used to show that the initial component of the response is mediated by substance P, whereas the long-lasting increase in blood flow is due to CGRP. These neuropeptides have also been shown to increase the protein permeability of vessels, which results in filtration of fluid from the blood vessels into the tissues (Olgart et al 1977, Kerezoudis et al 1993c).

Local intra-arterial infusion of synthetic CGRP and substance P has also been found to produce vasodilation in the dental pulp. The effect of CGRP was 10 times greater when it was administered after substance P rather than before it. Because the two peptides coexist in the same neurons, it is suggested that they both contribute to the vasodilation seen upon the antidromic stimulation of sensory nerves (Heyeraas et al 1994).

In one study, intermittent electrical stimulation every 5 minutes promptly increased PBF without causing significant changes in blood pressure (Fristad et al) (Fig 3-27a). PBF did not return to its original level within the 5-minute intervals between stimulation periods, but the increase in PBF following electrical stimulation gradually decreased and nearly approached control values by the end of the 4- to 5-hour stimulation period. The average blood flow increase at the start of the stimulations (the first three stimulations) was about 45.8% above the control PBF, whereas the blood flow increase in the subsequent stimulation periods were significantly less. Electrical stimulation of the mandibular first molar 3 days after inferior alveolar nerve axotomy resulted in no consistent increase in PBF (Fristad et al) (Fig 3-27b and 3-27c).

Kerezoudis et al (1993a) reported that the monopolar electrical stimulation of teeth (200 μA, 5 ms, 40 Hz, 1 s) in normal animals resulted in a transient 16% reduction in PBF, followed by a long-lasting 11% increase in PBF. When phenoxybenzamine or phentolamine were administered, the initial dip in PBF was reduced by 59%, and the subsequent increase was enhanced by 185% (Kerezoudis et al 1993a).

In another study, basal blood flow in the oral tissues remained unaffected after the infusion of CGRP, indicating




▲ **FIG 3-27** *(a)* Simultaneous recordings of systemic arterial pressure and PBF before, during, and after repeated electrical stimulation *(arrows)* of an innervated first molar in a rat. *(b and c)* The results 3 days after unilateral inferior alveolar nerve axotomy. $P_A$ = arterial pressure; PU = perfusion units. (Data from Fristad et al.)

that in the microvasculature of the oral tissues, basally released CGRP plays a minimal role in maintaining vasodilator tone (Kerezoudis et al 1994a). The administration of a neurokinin-1 receptor antagonist, SR 140.333 (300 mg/kg), resulted in a 17% decline in basal PBF, suggesting that the basal tonic release of these neuropeptides in dental pulp plays an important role in regulating the basal vasodilatory state and, therefore, pulpal homeostasis (Berggreen and Heyeraas 2000).

A fall in resting PBF and interstitial fluid pressure found after the administration of CGRP and substance P neuropeptide antagonists indicates that CGRP and substance P exert a basal vasodilator tone on precapillary pulpal vessels. It has also been reported that sensory neuropeptides exert a continuous influence on receptors in the vessels of normal, unstimulated pulp (Olgart and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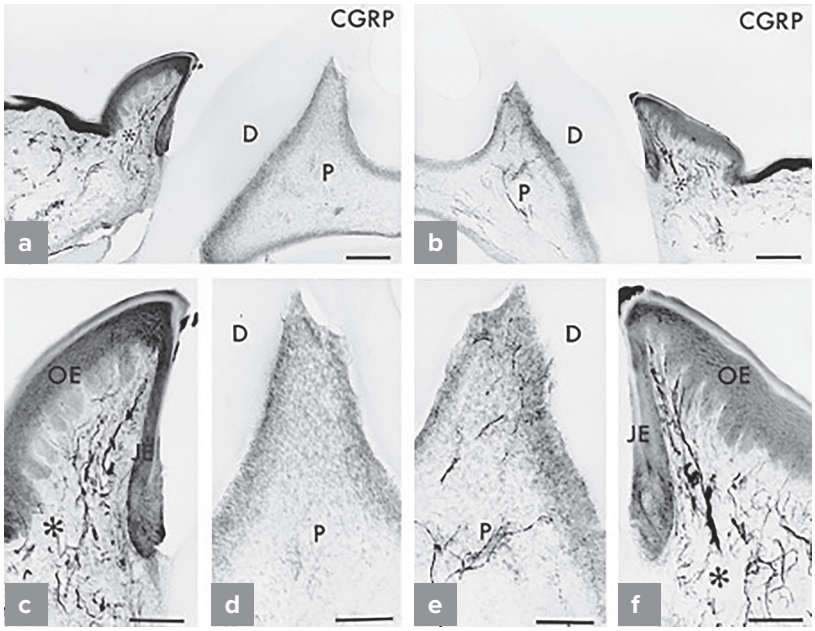
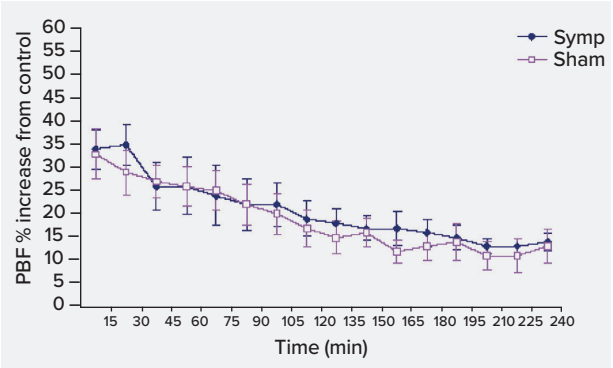



▶ **FIG 3-28** Immunohistochemical micrographs of *(a)* first molar pulp and gingiva showing CGRP-immunoreactive nerve fibers after intermittent long-lasting tooth stimulation and *(b)* the corresponding unstimulated control. *(c and d)* Higher magnification of the mesial papilla and dental pulp in *a*. *(e and f)* Higher magnification of the mesial papilla and dental pulp in *b*. Scale bars in *a* and *b* = 200 µm; scale bars in *c* to *f* = 100 µm; D = dentin; P = pulp; * = gingival papilla; OE = oral epithelium; JE = junctional epithelium. (Reprinted with permission from Fristad et al.)

Gazelius 1988, Olgart et al 1993). Electrical stimulation of the tooth empties the neuropeptide contents of the dental pulp nerve terminals, but this reaction is not seen in the gingiva (Fristad et al) (Fig 3-28). Furthermore, the spontaneous basal release of CGRP peptide has been reported to occur with the superfusion of bovine dental pulp in vitro (Hargreaves et al 1992, Hargreaves et al 1996).

In chronically sympathectomized animals, monopolar electrical stimulation of the tooth (200 µA, 5 ms, 40 Hz, 1 s) was found to cause an increase in PBF that was 250% greater than that observed in untreated animals. This increase in PBF was not enhanced with the administration of a-adrenergic blockers. Acute resection of the superior cervical sympathetic ganglion, however, did result in a 56% enhancement of the stimulation-induced increase in PBF. The increase in PBF was completely prevented by intravenous pretreatment with capsaicin.

These results suggest that the activation of sympathetic nerves exerts inhibitory effects on afferent nerve–induced vasodilation in rat incisor pulp unrelated to sympathetic vasoconstriction (Kerezoudis et al 1993a). Interestingly, Csillag et al showed that the average increase in PBF in rats was about 34% during the first 15 minutes of stimulation for both control animals and sympathectomized animals, indicating that sympathetic nerves were not involved in the increase in PBF resulting from the electrical stimulation of the teeth (Csillag et al) (Fig 3-29).




▲ **FIG 3-29** The effects of repeated electrical tooth stimulation and time on increases in PBF. Values represent the mean of three stimulation periods. *Filled circles* = sympathectomized animals; *open squares* = control animals. (Data from Csillag et al.)

### Capsaicin

One of the most recognized physiologic properties of capsaicin is its selective effects on the peripheral sensory nervous system, particularly its effects on primary afferent neurons. The activation of pulpal nociceptors with capsaicin triggers the peripheral release of neuropeptides such as substance P and CGRP (Hargreaves et al 1994).

Jacobsen and Heyeraas (1996) showed that repeated injections of capsaicin caused a consistent reduction in CGRP- and substance P–containing fibers in the jaws compared to the placebo-treated controls. However, there

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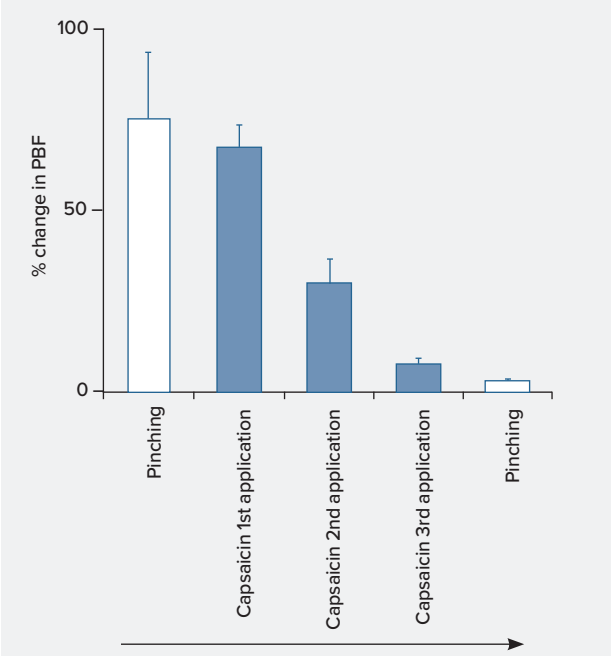
was not a complete loss of neuropeptides in the dental pulp. Local application of capsaicin in deep dentinal cavities induced a long-lasting PBF increase in only the control teeth. In addition, the onset of the PBF increase in the treated teeth was slower and the duration of the response significantly (60%) shorter than in control teeth. Intermittent grinding of the surface dentin instantly increased PBF in the control teeth by about 53%, whereas in denervated teeth, the response was delayed and significantly (70%) smaller. Deeper preparation produced responses of similar magnitude in control and denervated teeth (69% and 50%, respectively), but the onset was delayed in denervated teeth. Low-intensity ultrasonic stimulation caused a 38% increase in vasodilation in intact teeth but had no effect on denervated teeth. Sympathectomy did not influence stimulation-induced blood flow responses in the dental pulp, indicating that this effect is due to sensory nerves (Olgart et al 1991).

Capsaicin is known to have a highly selective desensitizing effect on polymodal C fiber nerve endings (Holzer). Sasano et al showed that responses in the dental pulp are caused by the activation of capsaicin-sensitive nerve fibers. Topical application of capsaicin to the ipsilateral gingiva elicited vasodilation in the dental pulp, and these vasodilator responses were remarkably reduced following repeated applications of capsaicin (Fig 3-30). These results suggest that axon reflex vasodilation in the dental pulp is mediated, at least in part, by polymodal C fibers.




▲ **FIG 3-30** Pinching-induced responses before and after three applications of 30 mM capsaicin. Increases in PBF were significantly reduced after capsaicin desensitization. The *arrow* shows the experimental sequence, with 25-minute intervals between applications. (Data from Sasano et al.)

### Nitric oxide

Nitric oxide (NO) is a water-soluble, unstable, free radical gas that reacts rapidly with oxygen to form nitrogen oxides. Normally, NO is produced in numerous tissues and is a mediator of cell-to-cell communication. It is involved in numerous processes, including vasodilation, inflammation, and neurotransmission. As mentioned previously, the basal rate of release of CGRP and, to a lesser extent, substance P regulates resting PBF. In ferret dental pulp, this resting vasodilator tone is due to the tonic release of CGRP, substance P, and NO (Kerezoudis et al 1995a, Berggreen and Heyeraas 1999).

Karabucak et al demonstrated that substance P induces endogenous NO production by activating NO synthase (NOS) in endothelial cells. The NOS inhibitor L-NAME completely blocks NO production. These results indicate that substance P–induced vascular relaxation can be mediated by inducing NOS, and subsequently NO, production in endothelial cells (Karabucak et al). The intra-arterial infusion of the CGRP receptor antagonist CGRP 8-37 resulted in a 32% reduction in basal PBF, measured with laser Doppler flowmetry (Karabucak et al).

NO differentially regulates basal blood flow and antidromic- or substance P–induced vasodilation in the microvasculature of the lips and dental pulp. The continuous infusion of L-NAME was found to reduce basal blood flow values in the pulp by 41% (Kerezoudis et al 1993b).

Berggreen and Heyeraas (1999) reported a basal resting vasodilator tone of sensory origin in the dental pulp due to NO maintaining a physiologic vasodilator tone in pulpal microvessels (Berggreen and Heyeraas 1999). Retrograde intra-arterial infusion of L-NAME significantly reduced laser Doppler flow and caused a consistent increase in interstitial fluid pressure. It was thus concluded that a resting vasodilator tone due to the release of CGRP, substance P, and NO exists in the ferret dental pulp. The sensory neuropeptides exert their effect predominantly on precapillary vessels, and NO exerts its effect predominantly on postcapillary vessels. The sensory neuropeptide CGRP is mainly responsible for the increase in blood flow and interstitial fluid pressure during tooth stimulation, whereas there was no evidence that NO participates in the vasodilation induced by tooth stimulation.

## Clinical Applications

There are several unique aspects of the pulpal circulatory system and PBF. This portion of the chapter describes the clinical implications of the vascular structure of the pulp and its various circulatory mechanisms.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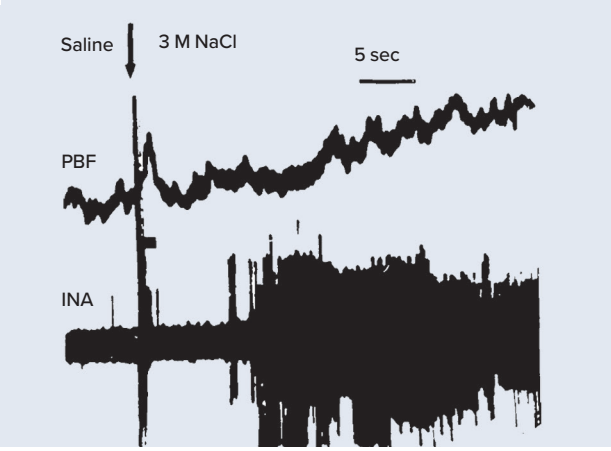
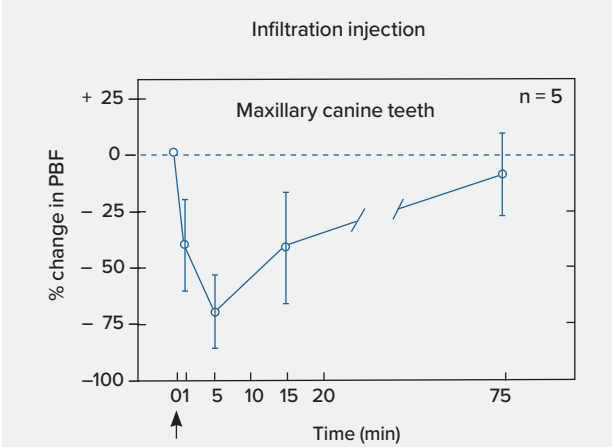



▲ **FIG 3-31** Polygraph tracing of simultaneous recordings of PBF and intradental sensory nerve activity in response to the application of 3 M sodium hypochlorite into a dentinal cavity in a cat canine tooth. The top tracing represents PBF measured with laser Doppler flowmetry, and the bottom tracing represents intradental sensory nerve activity measured with the intradental recording technique. INA = intradental sensory nerve activity. (Reprinted with permission from Kim et al 1984a.)




▲ **FIG 3-32** Effects of infiltration anesthesia (2% lidocaine with 1:100,000 epinephrine) on PBF in the maxillary canine teeth of dogs. The *arrow* indicates the time of injection. (Data from Kim et al 1984a.)

## Pain

Several study authors have stated that local microcirculatory changes may have profound effects on sensory nerve functions in the pulp (Edwall and Scott, Haegerstam, Olgart 1985, Närhi). Using measurements from a single nerve fiber, Närhi demonstrated that an increase in tissue pressure increases sensory nerve activity. Furthermore, simultaneous measurements of PBF and intradental sensory nerve activity in response to osmotic stimulation caused increases in both PBF and intradental sensory nerve activity, with PBF increases preceding increases in intradental sensory nerve activity (Kim 1990) (Fig 3-31). This sequence suggests that changes in PBF affect intradental sensory nerve activity.

## Inflammation

In 1971, Van Hassel showed that local inflammation in the pulp is accompanied by high pressure locally. Elevations in tissue pressure cause venous stasis and ischemia, but only within a limited volume of tissue, and Van Hassel concluded that the pathophysiology of the pulp is by a circumferential spread of inflammation and necrosis from the site of initial inflammation. Heyeraas, using the servo-nulling micropipette, simultaneously measured the tissue pressure in inflamed areas and the adjacent normal areas of the pulp, showing that the increased pressure (8–10 mmHg) in the inflamed area may be a local phenomenon that does not necessarily spread to the rest of the pulp (Heyeraas et al 1993, Heyeraas 1985). In addition, inflammation induced by deep caries in the coronal area of the tooth resulted in virtually no changes in blood flow in the apical area of the coronally inflamed pulp (Heyeraas et al 1993, Heyeraas 1985).

## Local anesthetics

Common local anesthetic agents contain adrenaline (epinephrine) in a 1:100,000 concentration. Hellner (1927) has suggested that a marked reduction in PBF resulting from the use of dental anesthesia could result in tissue injury (Kim et al 1984a). Olgart and Gazelius (1977) reported that the supraperiosteal injection of lidocaine containing epinephrine in the apical area of the tooth caused almost complete cessation of blood flow in the pulp.

Local anesthetic injections of 2% lidocaine with epinephrine 1:100,000 can significantly reduce PBF in teeth at or distal to the injection site. Kim et al (1984a) showed that PBF decreased to 60.6% that of the control PBF value 1 minute after the injection of local anesthetic solution (2% lidocaine with 1:100,000 epinephrine), and after 6 minutes, PBF was only 28.5% that of the control PBF (Fig 3-32). However, there were no significant changes in PBF in the control teeth throughout the experiments. Between 15 and 75 minutes were required for PBF to return to near-normal levels (Kim et al 1984a). Likewise, mandibular block anesthesia caused a decrease in PBF in both molar and canine teeth (Table 3-1). Within 6 minutes of injection, blood flow had decreased to

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47.2% of the PBF in the control molars and 33.2% of the PBF in the control canines (Kim et al 1984a).

Of the injection methods tested, the intraseptal and intraligamental injections caused the most severe reduction in PBF in molar teeth. This effect can probably be attributed to the epinephrine in the anesthetic solution. In contrast to the flow reduction seen with the intraseptal injection with 2% lidocaine with 1:100,000 epinephrine, PBF in molar teeth increased about 55% above that of the control when the intraseptal injection was made with 2% lidocaine alone (Kim et al 1984a). The blood flow had fallen to 9.6% of that in the control molar teeth and 30.2% in canines by 5 minutes after injection of the anesthetic solution (see Table 3-1). Even 65 minutes after injection, blood flow had only returned to 65.3% and 84.2% in molar and canine teeth, respectively (Kim et al 1984a). The effect of anesthetic agents on PBF has been confirmed in human subjects using laser Doppler flowmetry (Musselwhite et al).

The mechanism by which block anesthesia produces such a marked decrease in blood flow might be explained by the following hypothesis: Because the volumetric flow has a fourth-power relationship with the diameter of the vessel, according to the Poiseuille-Hagen law, a small change in vessel diameter produced by epinephrine near the inferior alveolar foramen would markedly affect the volumetric flow.

Because dental procedures are usually performed following the use of a local anesthetic, blood flow in the pulp may be compromised at the time of operation. Pashley (1979) has shown that the pulpal concentration of substances diffusing across the dentin depends in part on the rate of substance removal by the pulpal circulation. Thus, a significant reduction of blood flow resulting from the preoperative administration of a local anesthetic for a cavity or crown preparation could lead to a high concentration of irritants permeating the dentin. Thus, attempts should be made to maintain optimal PBF during dental restorative procedures.

**TABLE 3-1 PBF of the teeth measured 5 to 6 minutes after local anesthetic injections (2% lidocaine with 1:100,000 epinephrine)**

| | Canine teeth | | | Molar teeth | | |
|---|---|---|---|---|---|---|
| | Control (C) | Experimental (E) | E/C | Control (C) | Experimental (E) | E/C |
| Infiltration | 33.32 ± 6.40 | 7.72 ± 1.5 | 0.28 ± 0.08 | – | – | – |
| Mandibular block | 28.08 ± 5.39 | 9.88 ± 3.65 | 0.33 ± 0.09 | 26.30 ± 5.16 | 12.02 ± 2.21 | 0.47 ± 0.04 |
| Intraseptal | 27.36 ± 4.78 | 8.16 ± 1.45 | 0.30 ± 0.03 | 20.04 ± 3.81 | 1.76 ± 0.37 | 0.09 ± 0.01 |

## Conclusion

Advancements in electronics and radioisotope and laser technologies have allowed pulp physiologists to develop a better understanding of dental pulp circulation, including the roles played by neuropeptides and other mediators. The vitality of the dental pulp is dependent on adequate PBF and the intricate and complex mechanisms involved in maintaining it.

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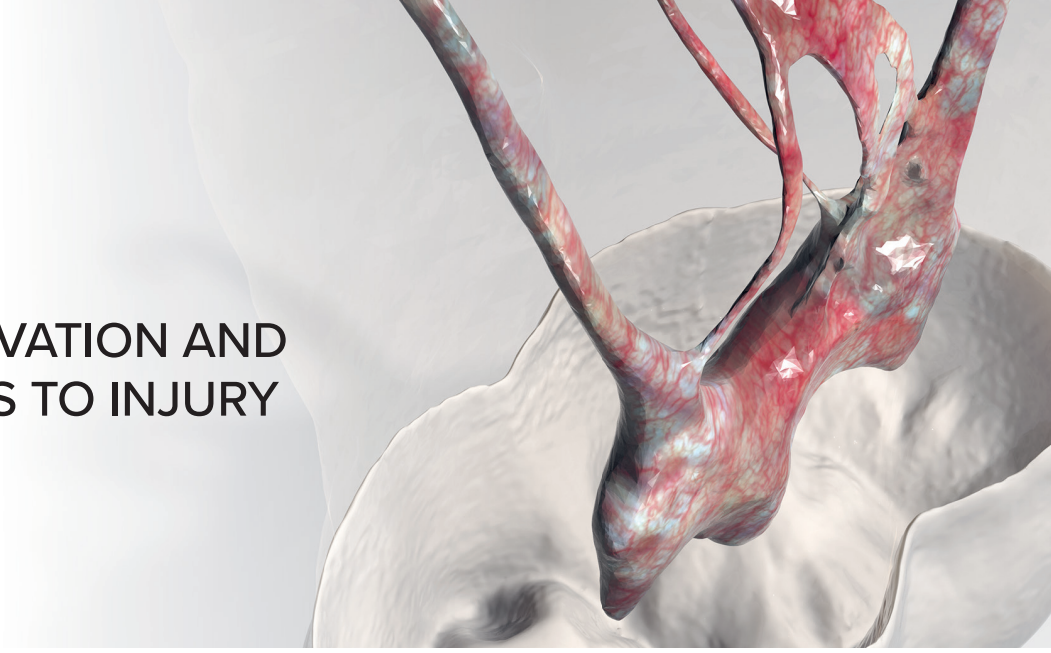

# 4

# DENTAL INNERVATION AND ITS RESPONSES TO INJURY

Javier Caviedes-Bucheli
Hugo Roberto Munoz

In ancient times, the dental pulp was considered to allocate supernatural entities or demons; therefore, the popular belief was that toothaches were punishments inflicted by superior powers (Hildebrand et al). In the 16th century, Vesalius published an outstanding anatomical finding in his book *De humani corporis fabrica*, where he described the tooth pulp as well as the presence of nerves by which he believed the teeth had the faculty of sensitivity (Hast and Garrison). Up to the present, patients have commonly referred to the dental pulp as the "nerve of the tooth," and some people consider root canal treatment the procedure for "killing the nerve." While it would be inappropriate to limit the dental pulp just to the tooth nerve, it is well known that the pulp is one of the most densely innervated body tissues. Over time, we have learned why the dental pulp is highly innervated, and the literature has showed us how this dense innervation plays a major role in *all* of the dental pulp functions, not only the sensory function, which is the most notorious and therefore most relevant for patients. Yet tooth innervation also controls many processes for dental pulp formation, nutrition, defense, and reparative functions.

This chapter presents an overview of dental pulp innervation, including its macro- and microanatomy, the pathways of somatosensory and autonomic dental innervation, and the classification of intradental axons that initiate the neural conduction through different nociceptors, mechanoreceptors, and proprioceptors. The close relationship that dental innervation has with the pulp microvasculature is essential to understand the roles that nerve fibers play in defensive neural responses to tooth injury provoked by caries, as well as by dental and restorative procedures. The interaction between nerve fibers and other cell populations of the pulp through the release of neurotransmitters and neuropeptides is also addressed.

Topics discussed in this chapter have a close relationship with those discussed in previous and later chapters. Final considerations are presented regarding how dental innervation has control over angiogenic mechanisms in the pulp, which are also important for the reparative function of the dental pulp.

## Macroanatomy of Dental Innervation

Innervation in the dental pulp is supplied by the somatosensory and autonomic nervous system. The latter is further subdivided into sympathetic and parasympathetic nervous systems. The somatosensory nerves supplying the pulp originate from the trigeminal ganglion, while the sympathetic nerves in the dental pulp come from the superior cervical ganglion. Controversies still exist regarding the origin of parasympathetic pulp fibers (Wakisaka).

### The somatosensory system: The trigeminal nerve

The trigeminal nerve is responsible for mediating all the sensory impulses from teeth to the central nervous system. It is the largest and most complex of the cranial nerves originating from the brainstem at the lateral surface of the mid pons, comprising a smaller motor and a larger sensory root (Shankland 2000). It is divided into three branches: ophthalmic (V1), maxillary (V2), and mandibular (V3). V1 supply sensation to the forehead, eyeball, eyelid, conjunctiva, lacrimal glands, nasal mucosa, and the skin of the nose (Shankland 2001a). V2 (Fig 4-1) exits the middle cranial fossa, through the foramen rotundum, traveling into the pterygopalatine fossa, where it subdivides to supply sensation to the maxillary teeth and their corresponding

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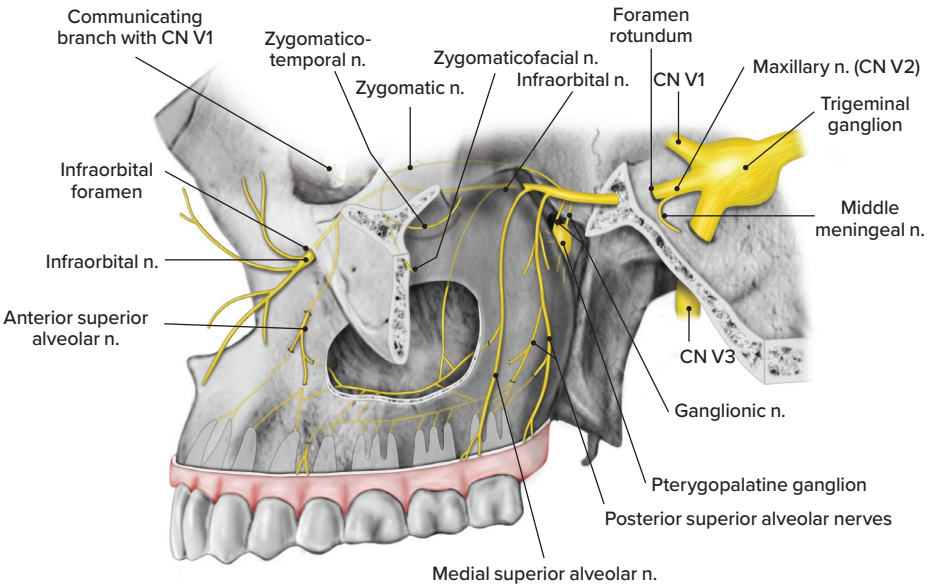
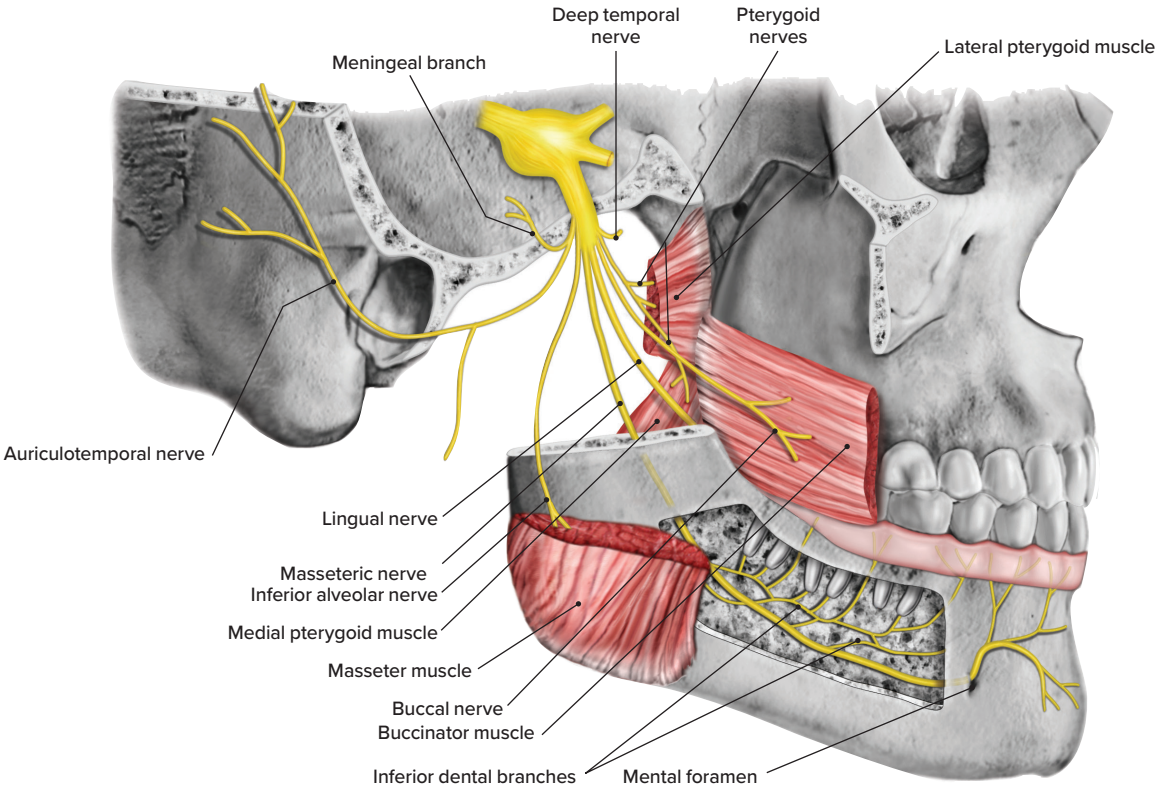



◀ **FIG 4-1** Schematic representation of the trigeminal maxillary nerve (V2) and its branches. Superior alveolar nerves provide all innervation to the dental pulps of the maxillary teeth. n. = nerve; CN V1 = ophthalmic nerve; CN V3 = mandibular nerve. (Reprinted with permission from Al-Faraje.)




▲ **FIG 4-2** Schematic representation of the trigeminal mandibular nerve (V3) and its branches, including the long buccal nerve, the inferior alveolar nerve, and the lingual nerve. The relationship between the inferior alveolar nerve and the auriculotemporal nerve as well as the masticatory muscle nerves can be observed. The multiple dental branches of the inferior alveolar nerve are responsible for providing all innervation to the dental pulps of the mandibular teeth. (Reprinted with permission from Al-Faraje.)

gingival tissues, the upper lip, lateral surface of the nose, skin of the cheek, nasal cavity, maxillary sinus, and the mucosa of the hard and soft palate (Shankland 2001b). V3 (Fig 4-2) exits the floor of the cranium through the foramen ovale into the infratemporal fossa and provides sensation to the mandibular teeth and their corresponding gingiva, the lower lip, chin, tongue, mucosa and skin of the cheek, temporomandibular joint (TMJ), auricle, muscles of the floor of the mouth, and the muscles of mastication (Shankland 2001c).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As stated above, dental pulps of maxillary teeth are innervated by the superior alveolar nerves, which are part of the V2 branch, while pulps of mandibular teeth are mainly innervated by the inferior alveolar nerves from the V3 branch. However, there are reports that the lingual, buccal, mylohyoid, and auriculotemporal (also derived from V3) nerves provide supplementary innervation to mandibular teeth (Carter and Keen, Frommer et al, Sutton, Rood 1976, Madeira et al, Wilson et al, Tong, Bennett and Townsend, Blanton and Jeske, Stein et al, Kim et al 2016). Cross innervation of the incisor teeth by the contralateral mental nerve has also been described (Pogrel et al). Knowledge of the pathways of the main and supplemental dental innervation is relevant for achieving satisfactory local anesthesia in clinical scenarios.

The trigeminal nerve is considered a mixed nerve. The afferent trigeminal fibers transmit signals from the face, nasal and oral cavities, and most of the scalp. The cell bodies of these sensory fibers are in the trigeminal ganglion (also known as the *Gasserian ganglion*), while cell bodies for proprioception (except mechanoreceptors from the periodontal ligament) and stretch receptors are located in the mesencephalic nucleus. Masticatory muscles are innervated by somatic efferent trigeminal fibers that originate from the motor nucleus of the trigeminal nerve located in the pons. Furthermore, the trigeminal nerve also contains visceral efferent fibers that merge with some facial or glossopharyngeal nerves and control lacrimal, salivary, and nasal mucosa glands (Rodella et al).

## The autonomic system: Sympathetic and parasympathetic innervation

The sympathetic system regulates many vital functions not necessarily related to hemodynamic regulation. One of the major roles of the sympathetic system is the maintenance of blood pressure. When a stimulus triggers increased sympathetic activity, an increase in blood pressure will take place by vasoconstriction of arterioles in most tissues. Although the sympathetic vasomotor control of the pulp may have local importance, it is insignificant for the circulatory homeostasis of the jaws (Olgart 1996).

The proportion of sympathetic fibers in relation to the total intrapulpal nerve supply was thought to be insignificant, constituting only up to 10% of the nerve fibers present in fully developed teeth (Christensen, Feher et al). However, there is now evidence that the sympathetic nervous system modulates immune function and constitutes an active interface between the central nervous system and the immune system (Madden et al, Tada, Straub et al, Downing and Miyan, Elenkov et al, Bedoui et al). The central nervous system not only receives messages from the immune system but also modulates immune function (Elenkov et al, Haug and Heyeraas).

Adrenergic vasomotor innervation in the human dental pulp has been demonstrated by means of histochemical and biochemical techniques (Anneroth and Norberg 1968, Pohto and Antila, Kukletova et al, Luthman et al 1992), showing that sympathetic fibers originate from the cervical sympathetic ganglion and may follow two pathways to reach the dental pulp. The main sympathetic pathway is one whereby the sympathetic fibers, after leaving the superior cervical ganglion, follow the common and external carotid arteries and then the maxillary artery to finally enter the pulp with the superior or inferior alveolar artery (Kerezoudis et al). The other pathway consists of a number of fibers that join the trigeminal nerve at its ganglion and follow the course of the sensory nerves to the teeth and adjacent tissues (Tønder and Naess, Matthews and Robinson, Marfurt et al, Kerezoudis et al).

After entering the pulp via the apical foramen, most of the sympathetic fibers form a plexus around blood vessel walls (Uddman et al 1984, Casasco et al 1995), while few others remain with no apparent connection with blood vessels (Luthman et al 1992, Heyeraas et al 1993, Uddman et al 1998). Interestingly, sympathetic nerve fibers rarely reach the odontoblastic and subodontoblastic layers (Wakisaka et al 1986), as they are more numerous in the radicular than in the coronal pulp (Uddman et al 1984, Haug et al).

The parasympathetic system mediates vasodilation and reflexogenic reactions in many body organs and tissues, including the skin, skeletal muscles, nasal mucosa, and salivary glands, among others. These actions are mediated by acetylcholine (Ach) and vasoactive intestinal peptide (VIP), which are produced by parasympathetic fibers associated with blood vessels. VIP has been suggested as a mediator of the noncholinergic (atropine-resistant) vasodilation observed in nasal mucosa and salivary glands of the cat (Lundberg et al 1981a, Lundberg et al 1981b, Lundberg et al 1981c).

The presence of parasympathetic nerves in dental pulp has been controversial, and the vasodilatory effect in the dental pulp blood vessel has been debated over time (Weiss et al, Edwall et al 1973, Cauvin and Kirkendol, Okabe et al, Caviedes-Bucheli et al 2006b). The VIP expression in a tissue could be used as a marker for parasympathetic neurons, as this neuropeptide is localized in these nerve fibers (Lundberg et al 1980, Lundberg et al 1981c). There is evidence of the presence of VIP-containing nerve fibers in the dental pulp of humans and other mammal species (Uddman et al 1980, Casasco et al 1990, Luthman et al 1992). Most of them are found in association with the pulpal vasculature, but some free fibers have also been observed in the stroma without relation to blood vessels (Wakisaka et al 1987). However,

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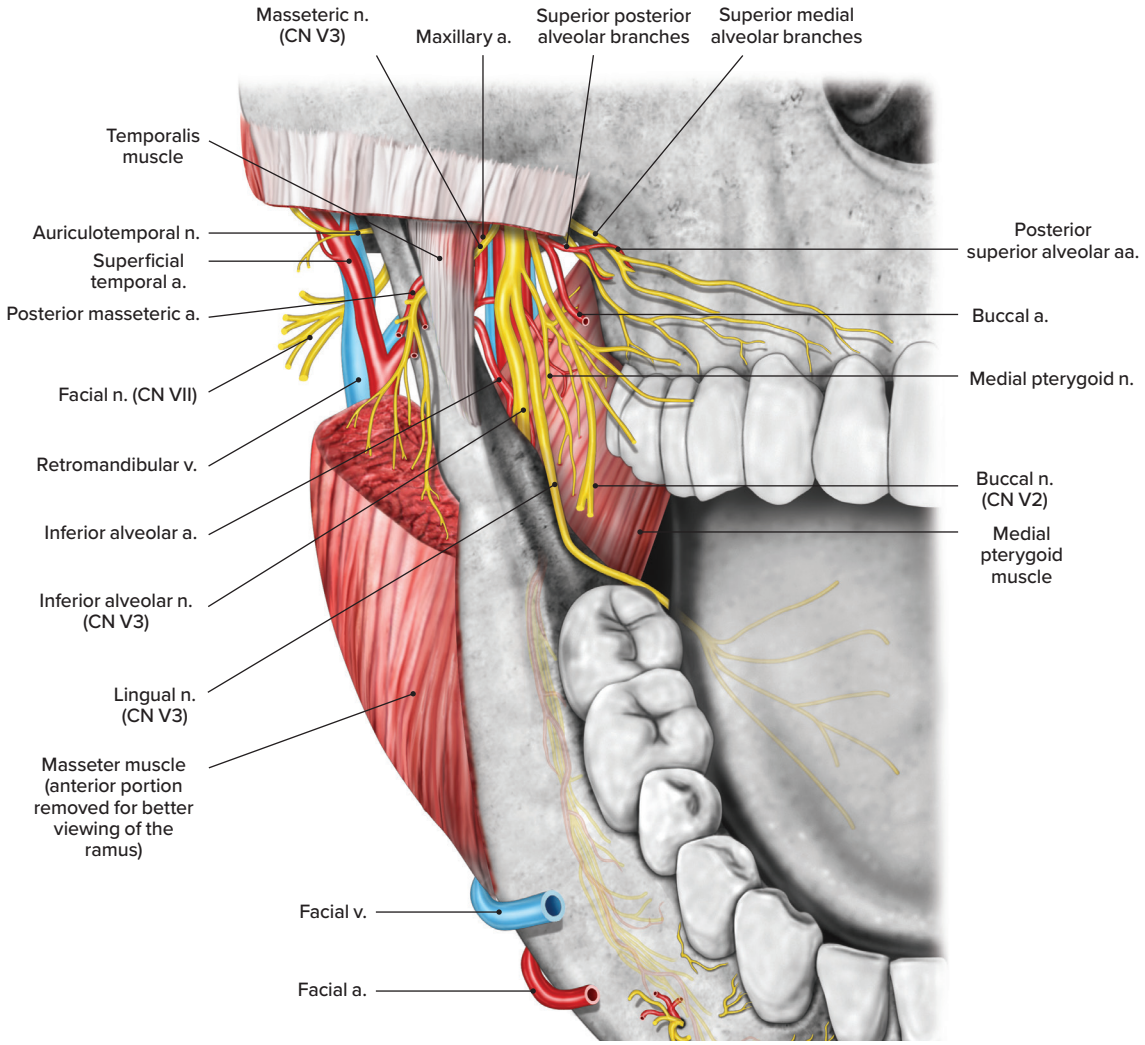


▲ **FIG 4-3** The trigeminal maxillary and mandibular nerves in conjunction with blood vessels that regulate blood flow to the skin, muscles, and teeth mainly by sympathetic and parasympathetic control. These autonomic nerve fibers run together with several branches of the trigeminal nerve. n. = nerve; a. = artery; aa. = arteries; v. = vein. (Reprinted with permission from Al-Faraje.)

no surgical denervation has resulted in complete depletion of VIP-containing fibers from the peripheral tissue, demonstrating that VIP does not originate from sensory or sympathetic nerves (McGregor et al, Atkinson and Shehab, Wakisaka et al 1986, Doughty et al 1991, Fristad et al 1998). Moreover, as VIP coexists with Ach in other tissues, it is suggested that these VIP-containing nerve fibers originate from a parasympathetic ganglion (Lundberg and Hokfelt). Postganglionic parasympathetic fibers originating in the otic ganglion (Fig 4-3) are distributed in several branches of the mandibular nerve, including the buccal, mylohyoid, and inferior alveolar nerves. They also innervate the small salivary glands in the buccal mucosa (Segade et al). The motor branches of the mandibular nerve to the masseter and deep temporalis muscles also contain postganglionic parasympathetic fibers from the otic ganglion (Zubair and Khan).

Therefore, all the prerequisites for parasympathetic control of pulpal blood flow (PBF) are present (nerves, mediators, and receptors). However, contradictory results are found in the literature. It has been published that there is an absence of parasympathetic vasodilation in the cat dental pulp (Sasano et al), but it has also been demonstrated that an intra-arterial injection of synthetic VIP in the picomolar range causes vasodilation in the cat pulp and that the adjacent gingiva is sensitive to even lower doses of VIP (Olgart et al 1988).


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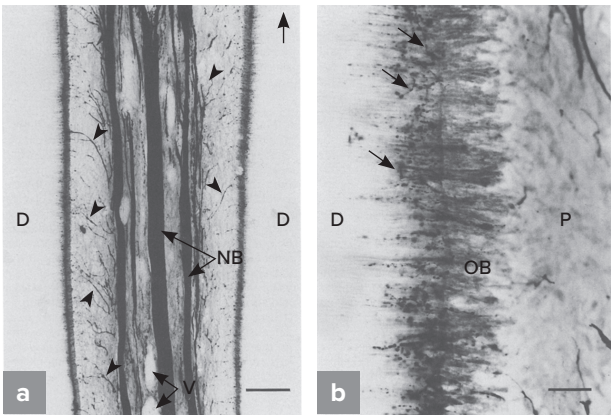


▶ **FIG 4-4** Light micrograph of longitudinal section of a human premolar immunostained with the neuron-specific protein gene product (PGP) 9.5 antibody. *(a)* Thick nerve bundles (NB) showing intense PGP 9.5 immunoreactivity run in the center of the radicular pulp. From these bundles, the branches appear to be distributed at regular intervals *(arrowheads)* and to extend toward the peripheral pulp. Antibody dilution = 1:15,000; D = dentin; V = blood vessel; scale bar = 200 µm; original magnification ×15. *(b)* A closer view of the dentin-pulp border zone. The nerve fibers pass through the odontoblast layer (OB) and enter the predentin (D). The *arrows* indicate the nerves taking a transverse course in the predentin. P = dental pulp; scale bar = 50 µm; original magnification ×75. (Reprinted with permission from Maeda et al.)

## Microanatomy (Histology) of Dental Innervation

Classical histologic studies have estimated the size and number of axons going into human teeth, with wide variations reported (due to species and age differences) in the number of myelinated and unmyelinated axons at the apical foramen and a greater abundance of unmyelinated axons (Graf and Bjorlin, Johnsen and Karlsson, Johnsen and Johns, Fried and Hildebrand 1981a, Fried and Hildebrand 1981b, Fried, Nair et al, Nair and Schroeder). Between 900 and 2,300 axons enter a human premolar tooth in the form of bundles (Reader and Foreman, Nair). The majority of axons present a diameter of less than 4 µm (Byers 1984). Degenerative studies have suggested that all of the myelinated axons are sensory (Arwill et al 1973, Feher et al), while only 5% to 13% of the unmyelinated axons are sympathetic, and the remaining are sensory (Fried and Hildebrand 1978, Noga and Holland, Nair). After passing the apical foramen, the sensory nerve fiber bundles accompany blood vessels along the long axis of the tooth, and some single fibers are observed in the peripheral area of the radicular pulp (Fig 4-4). When approaching the cervical portion of the root, the nerve bundles gradually branch along with the ramification of blood vessels and run to the peripheral area, reaching the cell-rich zone and forming a subodontoblastic plexus. At this plexus, some of these fibers branch again and terminate among the odontoblasts. There are also fibers that reach the odontoblast layer directly from the central pulp without being part of the subodontoblastic plexus (Itoh).

After the nerve bundles reach the coronal portion of the tooth, they branch into smaller fiber bundles that extend peripherally to cover all the surfaces of the pulp chamber (Fig 4-5). These nerve fibers also form a subodontoblastic nerve plexus adjacent to the cell-rich zone, also known as the *plexus of Raschkow*, which runs parallel to the odontoblast layer. The nerve endings are most numerous near the tip of the pulp horn and decrease toward the cervical zone (Byers 1994, Ramieri et al). An immunocytochemical study revealed a dense nerve supply in the radicular dentin-pulp border zone, identical to that in coronal regions, concluding that dense innervation in radicular pulp is one of the factors responsible for dentinal hypersensitivity (Maeda et al). Silver impregnation studies as well as autoradiographic studies with tritiated proline injections have shown that the plexus of Raschkow is formed by sensory nerve fibers originating at the trigeminal ganglion (Pimenidis and Hinds). Fibers from this subodontoblastic plexus cross the cell-rich and cell-free zones, reaching the odontoblast layer and in some cases going into the predentin, where axons could be easily identified by their distinctive vesicles and mitochondria (Byers et al 1982). Some pulpal axons lose the Schwann cell ensheathment and the basal lamina and appear to form terminals in the marginal plexus (Holland 1980). The intrapulpal axons have thinner and shorter myelin sheaths than similarly sized axons in extrapulpal nerve trunks (Hildebrand et al).

Small unmyelinated nerves in the odontoblast region and within dentinal tubules have been observed in ultrastructural studies (Arwill 1967, Frank, Avery et al). Although the extent of the nerve fibers into the dentin has been controversial, it is widely accepted that in some cases fibers extend into the calcified dentin through dentinal tubules closely associated with the odontoblastic process (Fearnhead, Corpron and Avery, Dahl and Mjör, Roane et al, Johnsen and Karlsson, Byers 1984). Because intradentinal axons extend up to 100 to 150 µm into the tubules, the innervation of dentin appears to be restricted to the

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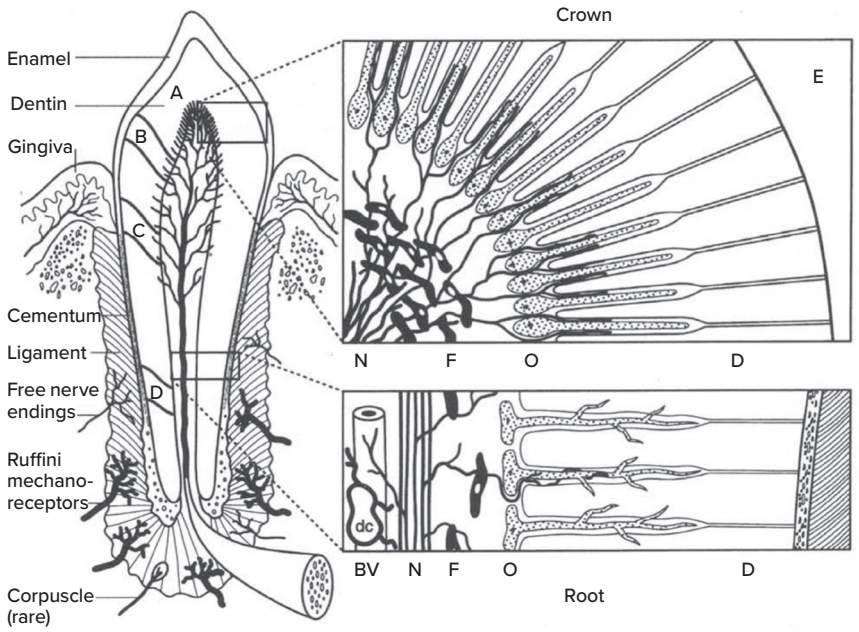
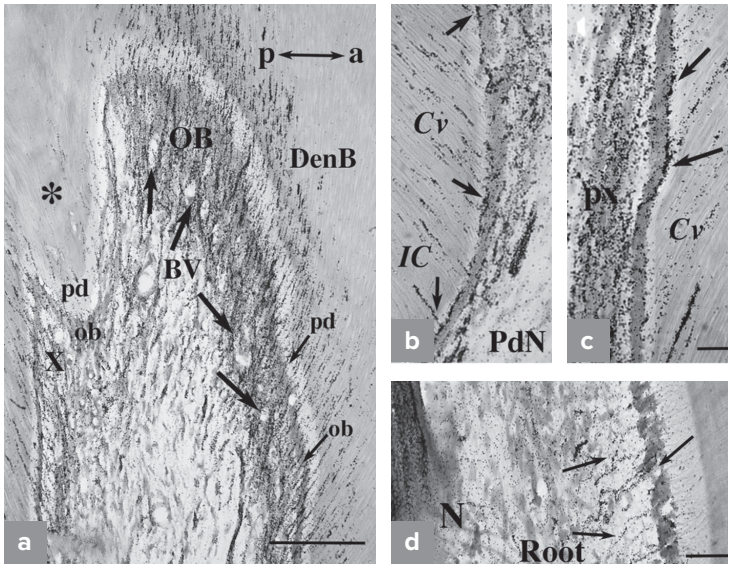



◀ **FIG 4-5** Schematic diagram of a mature erupted tooth and its dentinal, pulpal, and periodontal innervation, with various structures indicated. The labeled dentinal zone A has more than 40% of dentinal tubules innervated at the tip of the pulp horn, with decreasing percentages of innervated tubules in zones B (4.1% to 8.3%), C (0.2% to 1.0%), and D (0.02% to 0.2%). The higher-magnification right panels show differences in intradentinal nerve (N) incidence, pulpal fibroblasts (F), odontoblast morphology (O), dentin (D), and enamel (E) for the crown and root. Perivascular dendritic cells (dc) and vascular innervation (BV) are shown in the root diagram. (Reprinted with permission from Byers and Närhi).

inner part (Byers 1985, Närhi 1990), and no nerve fibers have been observed in the peripheral dentin or close to the enamel junction (Tsatsas and Frank). A single pulpal stem axon may innervate more than 100 dentinal tubules (Gunji, Byers 1985, Byers 1994). A recent autoradiography study showed a massive number of innervated dentinal tubules (Fig 4-6) (Byers and Calkins), and axonal transport showed that dentin sensory endings are in a majority of dentinal tubules beneath each cusp tip (Byers and Dong). Almost the entire dentin innervation is sensory (Arwill et al 1973, Avery et al, Byers and Dong, Marfurt and Turner), but it is also possible to observe a few sympathetic endings (Byers 1994). Most of these sympathetic axons form plexuses around pulpal blood vessels with α- and β-adrenergic receptors (Tønder and Naess), while approximately 30% of them reach the odontoblastic layer, and only few of them enter the dentin (Fig 4-7) (Avery, Avery et al, Byers 1984).

Sensory terminals in the dental pulp are organized into beads that contain clear and dense core vesicles, mitochondria, smooth endoplasmic reticulum, and cytoskeletal




◀ **FIG 4-6** Autoradiograph images of the pulp of a rat molar. *(a)* Dentin-pulp zone near the pulp horn shows elongated odontoblasts (OB) and increased vascularity (BV, *arrows*) on the anterior side of the tertiary dentin (*). The odontoblast layer becomes cuboidal and separated by a cell-free zone from the plexus of Raschkow (ob, *arrow*) closer to the root. Scale bar = 100 µm. *(b and c)* Two different views of cervical regions (Cv) and intercusp (IC) show predentin nerve (PdN) networks parallel to the odontoblasts in the narrow predentin region with some axons branching into tubules of calcified dentin *(arrows)*. *(d)* The root pulp below the cusp had detectable thin axons *(arrows)* in pulp between the main root nerve (N) and the odontoblast layer, with some extensions partway into predentin. Scales bars in *c* and *d* = 20 µm. (Reprinted with permission from Byers and Calkins.)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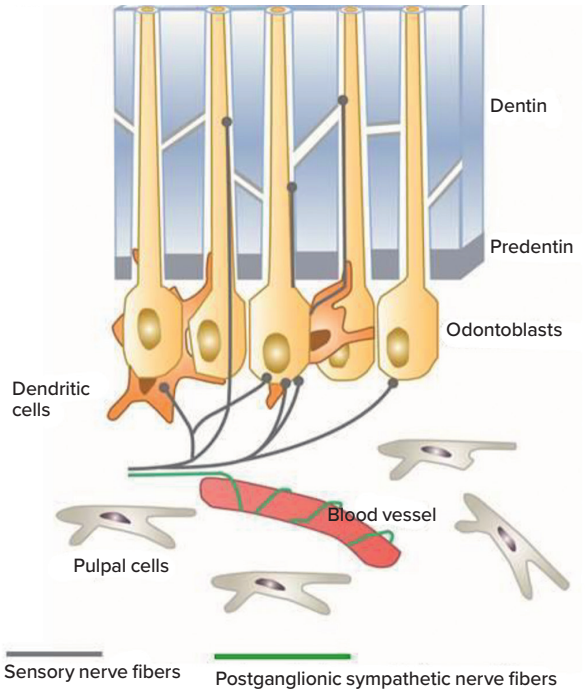
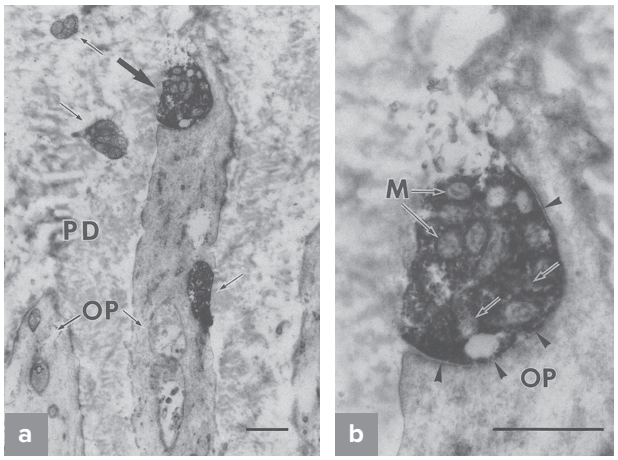



▲ **FIG 4-7** Schematic representation of the odontoblasts and their relationship to the dentin and nerve fibers, showing that almost all of the fibers reaching the dentin area are of sensory origin, while autonomic fibers remain mostly surrounding blood vessels. (Reprinted with permission from Solé-Magdalena et al.)




▲ **FIG 4-8** Immunoelectron microscope images of nerve terminal in human radicular predentin (PGP 9.5 immunoreaction). *(a)* Neural elements filled with electron-dense precipitates *(small arrows)*. Two nerve terminals are seen in contact with one odontoblast process (OP). Scale bar = 1 pm; original magnification ×5,000. *(b)* High-power electron micrograph of the nerve terminal indicated by the *large arrow* in *a*. The terminal is filled with reaction products for PGP 9.5, but mitochondria (M) and vesicles *(arrows)* are negative. Note the absence of a synaptic structure between the nerve terminal and the odontoblast cell process *(arrowheads)*. Antibody dilution = 1:30,000; scale bar = 1 pm; original magnification ×15,000. (Reprinted with permission from Maeda et al.)

elements. They are in proximity to the blood vessels in the plexus of Raschkow. Terminals in dentinal tubules are also beaded and are thinner than the odontoblast process and may coil around it (Byers 1984, Hildebrand et al). At the odontoblastic layer, initial electron microscopic studies had suggested that gap junctions occur between odontoblasts and sensory axons (Holland 1975, Matthews and Holland). However, gap junctions between axons and odontoblasts have not been found in following studies (Holland 1980, Holland 1981), and axon identification by axonal transport labeling testing has failed to find axon-odontoblast gap junctions, only associations between the two cells, with apposed membranes and a cleft between them (Byers 1979, Byers et al 1982). Moreover, axo-axonic appositions could also be found in the plexus of Raschkow, the cell-free zone, predentin, and dentin, with axons separated only by a 5- to 10-nm extracellular space, which may be the structural basis for coupling between axons in teeth (Fig 4-8) (Matthews and Holland, Byers 1984).

A recent study has demonstrated that a single molar may be targeted by a dedicated population of about 50 specialized trigeminal neurons. By means of transcriptomic profiling, it was found that the majority of the expressed markers were for fast-conducting neurons, with about two-thirds containing nociceptive markers (Emrick et al).

# NEUROPHYSIOLOGY OF THE DENTAL PULP

## Classification and Roles of Peripheral Nerve Fibers

Electrophysiologic investigations on intradental nerves have shown that there are two groups of fibers—fast- and slow-conducting—and that these groups differ functionally (Närhi et al 1982b, Närhi et al 1982c, Närhi et al 1983,

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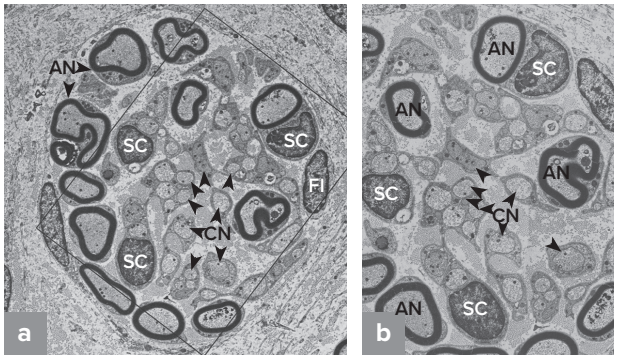
**TABLE 4-1 Axon classification according to diameter and conduction velocity**

| Fiber type | Axon diameter (μm) | Conduction velocity (m/s) | Myelin sheath | Localization | Presence in dental pulp | Function |
|---|---|---|---|---|---|---|
| Aα | 13–20 | 80–120 | Yes | Major muscles | No | Motor, proprioception |
| Aβ | 6–12 | 35–75 | Yes | • Cutaneous mechanoreceptors<br>• Secondary receptors of muscle spindle | Yes | Touch, pressure |
| Aγ | 5–8 | 4–24 | Yes | Muscle spindles | No | Motor |
| Aδ | 1–5 | 5–35 | Yes | • Free nerve endings<br>• Nociceptors<br>• Cold thermoreceptors | Yes | Pain, temperature |
| B | 1–3 | 3–15 | Yes | Preganglionic sympathetic fibers | No | Autonomic |
| C | 0.2–1.5 | 0.5–2.0 | No | • Trigeminal dorsal root fibers<br>• Nociceptors<br>• Heat thermoreceptors<br>• Postganglionic sympathetic fibers | Yes | Pain, temperature, itch |

Närhi and Haegerstam, Virtanen et al). The conduction velocity of nerve fibers depends on their axon diameter, which is the main criterion used to classify peripheral fibers (Table 4-1). A- and B-fibers are myelinated and therefore thicker, as their diameters include the thickness of myelin sheaths. A-fibers can be divided into four subtypes (in decreasing size order): α (alpha), β (beta), γ (gamma), and δ (delta). Finally, C-fibers have the smallest and nonmyelinated axons (Nair). There are many Aδ- and C-fibers in the tooth pulp, with the Aδ-axons ending mostly in the inner third of the dentinal tubules and the C-fibers ending mostly in the pulp itself (Hildebrand et al, Byers and Närhi, Byers et al 2003, Paik et al 2009).

Human tooth pulps contain around 80% to 90% C-fibers, while most myelinated pulpal axons (93%) belong to the Aδ size range (Reader and Foreman, Johnsen et al, Nair et al). Only a few myelinated pulpal axons (7%) belong to the Aβ size range (Fig 4-9) (Fried and Hildebrand 1981b, Cadden et al, Dong et al 1985, Nair et al). These findings suggest that different types of nerve fibers function when different stimuli are applied to human teeth; for example, sharp and well-localized pain and dull, poorly localized pain are the result of activation of Aδ- and C-fibers, respectively (Mumford and Bowsher, Price and Dubner, Torebjork and Hallin, Närhi et al 1992).

In the extradental main nerve trunk, a significant portion of the afferent pulpal axons are large and myelinated, suggesting that the Aδ- and C-fibers predominating inside the pulp constitute terminal segments of large, myelinated axons. This is supported by the observation




◀ **FIG 4-9** Transmission electron micrographic montage of an intraradicular bundle of nerve fibers containing both myelinated (AN, A-fiber) and nonmyelinated (CN, C-fiber) axons. *(a)* Note the absence of perineurium around the axon bundle (original magnification ×6,800). *(b)* Magnified view of rectangular block in *a* showing the fine structural details of nonmyelinated nerve fibers (CN) distributed among the myelinated axons (AN). Note the wide variation in size of the nonmyelinated axons (original magnification ×11,500). (Reprinted with permission from Nair.)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that pulpal axons have higher conduction velocities along extrapulpal than along intrapulpal segments (Lisney, Cadden et al, Hildebrand et al). Moreover, the presence in unmyelinated fibers of neurofilament heavy (NFH), a protein commonly expressed within sensory nerves that give rise to myelinated fibers, supports the notion that unmyelinated pulpal afferents originate from myelinated axons (Henry et al). Most intrapulpal axons exhibit conduction velocities characteristic of Aδ- and C-fibers and have different functions. The Aδ-fibers have a lower electrical threshold than the C-fibers, and therefore they respond to stimuli that normally do not activate C-fibers (Olgart 1974). Aδ-fibers are excited by hydromechanical stimuli at the dentinal surface or at the pulp-dentin border (eg, drilling or air drying of dentin) and mediate acute, sharp pain. C-fibers are activated by stimuli strong enough to reach the central pulp (ie, excessive heating or cooling of a tooth) and mediate dull and burning pain (Närhi 1985b, Markowitz and Kim), suggesting that they are nociceptive (Hildebrand et al).

Contrary to A-fibers, intradental C-fibers respond to the application of bradykinin and histamine (Jyvasjarvi and Kniffki 1989; Närhi 1990). Aβ-fibers respond to dentin probing and drilling like Aδ-fibers do, suggesting that they belong to the same functional group (Närhi 1985a, Närhi 1990). However, Aβ-axons mediate nonpainful sensations, such as mastication reflexes (Närhi et al 1982a, Närhi et al 1982c, Cadden et al, Virtanen et al, Dong et al 1985, Olgart et al 1988). Certain functional characteristics of intradental A- and C-fibers may provide some explanation for the variability of symptoms in pulpitis. It seems that unmyelinated C-fibers are more resistant against pressure elevation and hypoxia than myelinated A-fibers (Närhi 1985b, Närhi et al 1992), allowing them to transmit nociceptive impulses even when there is damage to the pulp tissue that might cause myelinated axons not to work because of the anoxic conditions (Nair). Thus, when an injury results in interruption to the pulp microcirculation, the C-fibers continue to function for a longer time compared to the A-fibers because the latter have been inactivated or infracted (Närhi 1985b, Caviedes-Bucheli et al 2006a). The ability of C-fibers to survive and function under extreme conditions could explain why some teeth may not respond to cold testing but manifest pain during root canal preparation (Figdor).

## Nociceptors, Mechanoreceptors, and Proprioceptors

The three peripheral sensory neuron subtypes—nociceptors, mechanoreceptors, and proprioceptors—localize to the dorsal root ganglia and transmit sensations such as pain, temperature, pressure, and limb movement/position (Saito-Diaz et al). Nociceptors respond to noxious heat and chemical stimuli, conducting pain sensations. They usually are small to medium in diameter, thinly myelinated or unmyelinated, and marked by the neurotrophin tropomyosin receptor kinase (TRK)-A (Lallemend and Ernfors). Normally, pain is felt when signals generated by noxious stimuli in the thinly myelinated Aδ- or the unmyelinated C-fiber nociceptive afferents reach the brain (Fried et al 2011). Mechanoreceptors conduct touch sensations and are of a larger diameter than myelinated neurons expressing TRK-B/TRK-C (Poole et al). Proprioceptors are large-diameter TRK-C+ neurons that conduct limb movement and position (Lallemend and Ernfors). Despite previous reports that the dental pulp was provided only with nociception capabilities, more currently it has been suggested that teeth are also innervated with neurons that may have a more discriminative role (Won et al, Emrick et al).

It has been reported that tooth pulp neurons also respond to thermal stimulation (Hu and Sessle). A recent study investigated the response properties of trigeminal neurons to noxious heat and/or cold stimuli applied to the teeth; 15% of the neurons that responded to electrical stimulation of the pulp also responded to noxious thermal stimulation of the tooth (Ahn et al 2012). Although some of the few Aβ-fibers present might be nociceptive (Djouhri and Lawson), most of them are considered to be low-threshold mechanoreceptors (LTMs) that conduct a nonpainful tingling when the tooth is stimulated electrically at liminal current strength; this tingling sensation is known as "prepain" (Dong et al 1993). It has been proposed that these peripheral afferent nerves be called "algoneurons" (Fig 4-10) (Fried et al 2011).

The mechanosensor Piezo2 has been recently characterized; it is preferentially found in medium- to large-sized A-fibers and is crucial for light touch sensation (Szczot et al). Because most dentinal tubules and the outermost pulp are innervated primarily by pulpal A-fibers (Jyvasjarvi and Kniffki 1987), it is suggested that Piezo2 is expressed in dental primary afferent (DPA) neurons to mediate mechanotransduction (Fig 4-11). Research on the neurochemical profile of Piezo2 messenger RNA (mRNA) suggests that a subpopulation of DPA neurons express mechanosensitive properties via expression of Piezo2 and that these neurons are functionally distinct from conventional nociceptors (Nguyen et al, Won et al). Transcriptomic profiling with retrograde tracing has proved that about a quarter of the labeled neurons of the pulp do not seem to be nociceptors, implying that they have a role in discriminative touch. It is suggested that dental neurons are capable of providing mechanosensitive information to start a rapid behavioral response and protect teeth from damage. Moreover,

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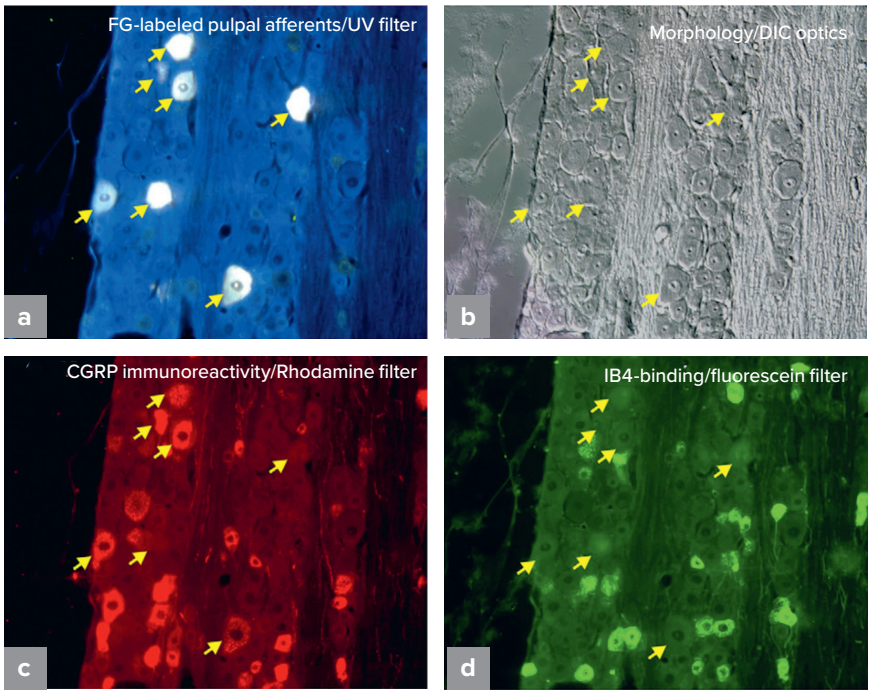


**FIG 4-10** Many dentinal afferents express neurotransmitters typical of nociceptors. Images show dentinal afferent neurons in the rat trigeminal ganglion (TRG) labeled with fluorogold (FG) that was applied to the dentin in the floor of shallow cavities in the maxillary first and second molars. After 4 weeks, the TRGs were fixed, removed, and processed for immunocytochemistry. *(a) Arrows* indicate FG-labeled neurons of medium to large size (intrinsic FG fluorescence). *(b)* The same field in Nomarski differential interference contrast (DIC) illumination. Many small-diameter neurons are present alongside medium and large neurons. *Arrows* point to the same neurons indicated in *a*. *(c)* The same field immunolabeled using an antibody that recognizes calcitonin gene-related peptide (CGRP). Many small afferent neurons are labeled as well as the medium and large dentinal afferents labeled with FG in *a (arrows)*. *(d)* None of the FG-labeled dentinal afferents binds IB4, a lectin associated with a subpopulation of small nociceptive neurons, so-called "algoneurons." (Reprinted with permission from Fried et al 2011.)

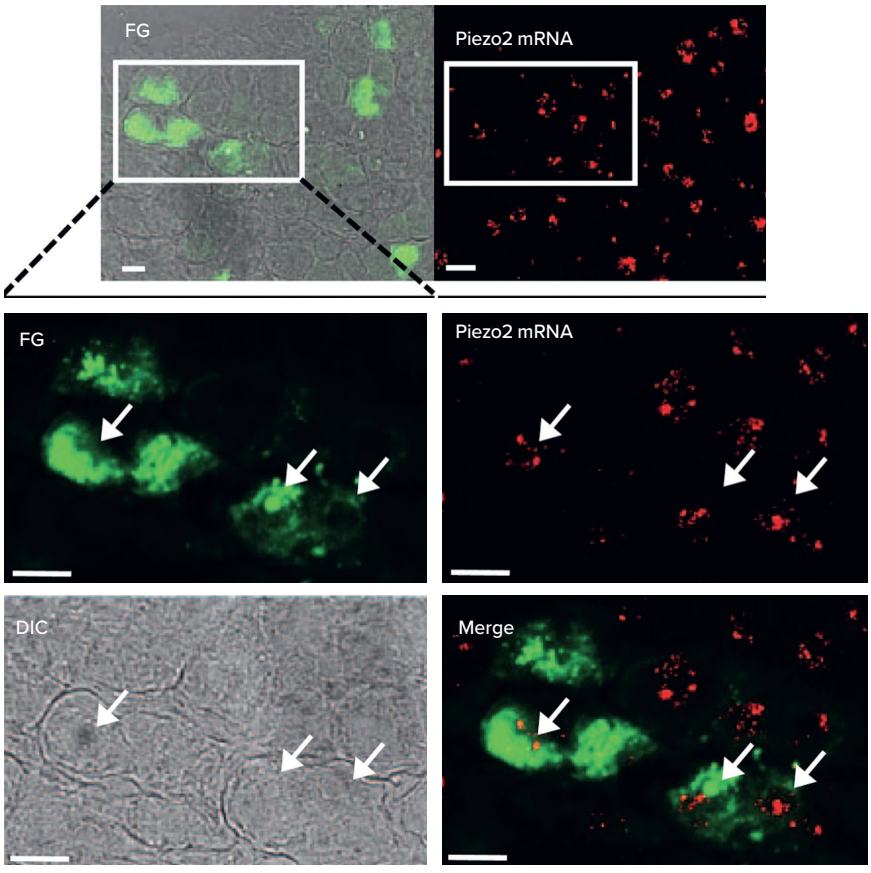


**FIG 4-11** Micrographs of FG-labeled DPA neurons *(green)* in whole trigeminal ganglion section and Piezo2 mRNA *(red fluorescent)*. *Arrows* highlight DPA neurons positive for Piezo2 mRNA. DIC = differential interference contrast image; scale bar = 25 µm. (Reprinted with permission from Won et al.)



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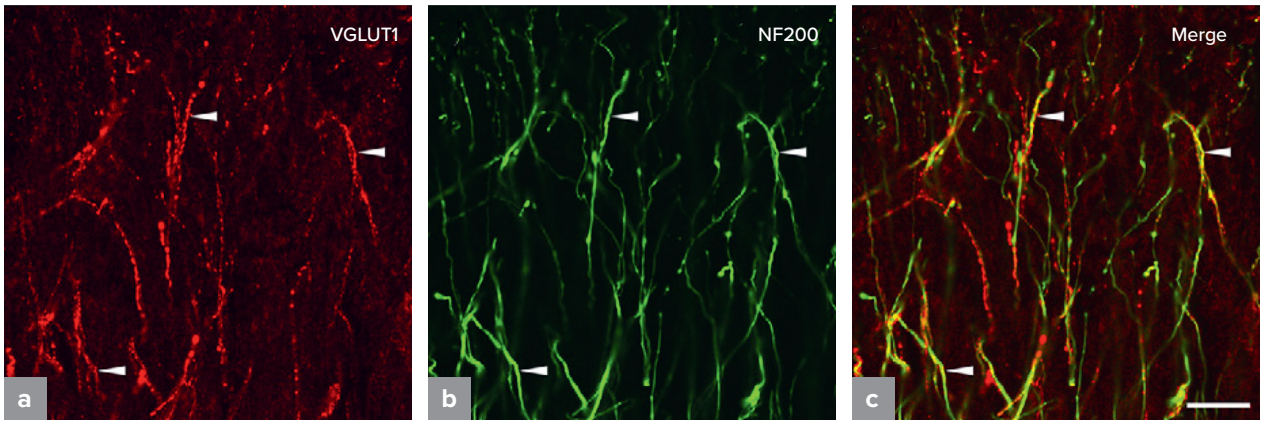


▲ **FIG 4-12** Double immunofluorescent staining for VGLUT1 and NF200 (a marker for neurons) in the coronal portion of human dental pulp. *(a to c)* VGLUT1-immunopositive axons co-stained for NF200 *(arrowheads)*, indicating that VGLUT1 was expressed in pulpal axons. Scale bar = 20 µm. (Reprinted with permission from Paik et al 2012.)

almost all retrograde labeled neurons, including nociceptors, expressed Piezo2 mechanosensor (Emrick et al).

Proprioception relies on populations of mechanosensory neurons distributed throughout the body, which are collectively known as *proprioceptors* (Tuthill and Azim). Proprioception has been considered to be a function of masticatory muscles, TMJs, and outer tooth structures such as the periodontal ligament. Traditionally, proprioception had been associated with larger myelinated Aα-fibers, which present immunoreactivity for neurofilament protein (NFP), calcium-binding protein parvalbumin (PV), and synaptophysin. These proteins are the usual markers for proprioceptors (Kim et al 2017, Byers and Cornel). There is evidence that intradental, low-threshold, mechanosensory, nonpainful systems could provide unperceived proprioception during normal teeth functions such as chewing, biting, gritting, grinding, grasping, or bruxing via Aδ and Aβ fast-conducting somatosensory axons (Cadden et al, Dong et al 1985, Dong et al 1993, Olgart et al 1988, Kubo et al). These neurons respond vigorously to tooth vibration requiring low-threshold, intradental mechanisms (Paphangkorakit and Osborn, Robertson et al, Byers and Cornel). A key feature of proprioception is its unperceived nature, which is also found for Aβ intradental axons where human perceptions are difficult to elicit or absent (Ikeda and Suda, Robertson et al, Byers 2019), but it might also contribute to tooth pain and jaw opening reflexes in order to protect the enamel when chewing forces reach dangerous levels (Dong et al 1985, Fried et al 2011).

## Neurotransmitters

Glutamate is considered one of the classical neurotransmitters acting on sensory fibers. It is produced by deamination of glutamine in the cell bodies of neurons at the dorsal root ganglia and trigeminal ganglion and then transported to the peripheral axons in dental pulp (Miller et al), where it can be released by electrical, chemical, or thermal stimuli (Jin et al). Glutamate release is also increased in the presence of injury or inflammation (Cho et al). The expression of glutamate receptors (GluR) and molecules involved in glutamate release in pulpal myelinated and unmyelinated axons has been described (Kim et al 2009, Paik et al 2012), suggesting that glutamate signaling plays an important role in acute and pathologic dental pain by either autocrine or paracrine activation (Miller et al, Bae and Yoshida).

Several studies have demonstrated the role of glutamate on nociception. It has been shown that vesicular glutamate transporters (VGLUT)-1 and -2 are expressed in several pulpal axons (Fig 4-12), suggesting that VGLUT1- and VGLUT2-mediated glutamate signaling is involved in pulpal nociception (Paik et al 2012). The application of L-glutamate to the rat skin excites Aδ- and C-fibers, inducing their sensitization to heat and generating thermal hyperalgesia (Du et al). Also, injecting glutamate into the human masseter muscle or into the TMJ causes acute pain and mechanical allodynia (Svensson et al 2003, Svensson et al 2005, Alstergren et al). VGLUT1 expression in pulpal axons and trigeminal ganglion neurons remains unchanged following pulpal inflammation, suggesting that the VGLUT1-mediated glutamate signaling may be involved in the mechanisms of acute nociceptive pain but not in the mechanisms of inflammatory pulpal pain (Yang et al). Glutamate release may also evoke neuropeptides from pulpal axons, which are known to induce neurogenic inflammation and therefore contribute to sensitization of the pulpal nociceptive axons (Jackson and Hargreaves).

Odontoblasts also have the capability to initiate glutamate signaling, as they contain high levels of glutamate that exceed their metabolic needs; increased expression of the mGluR5 receptor has also been observed in pulpal

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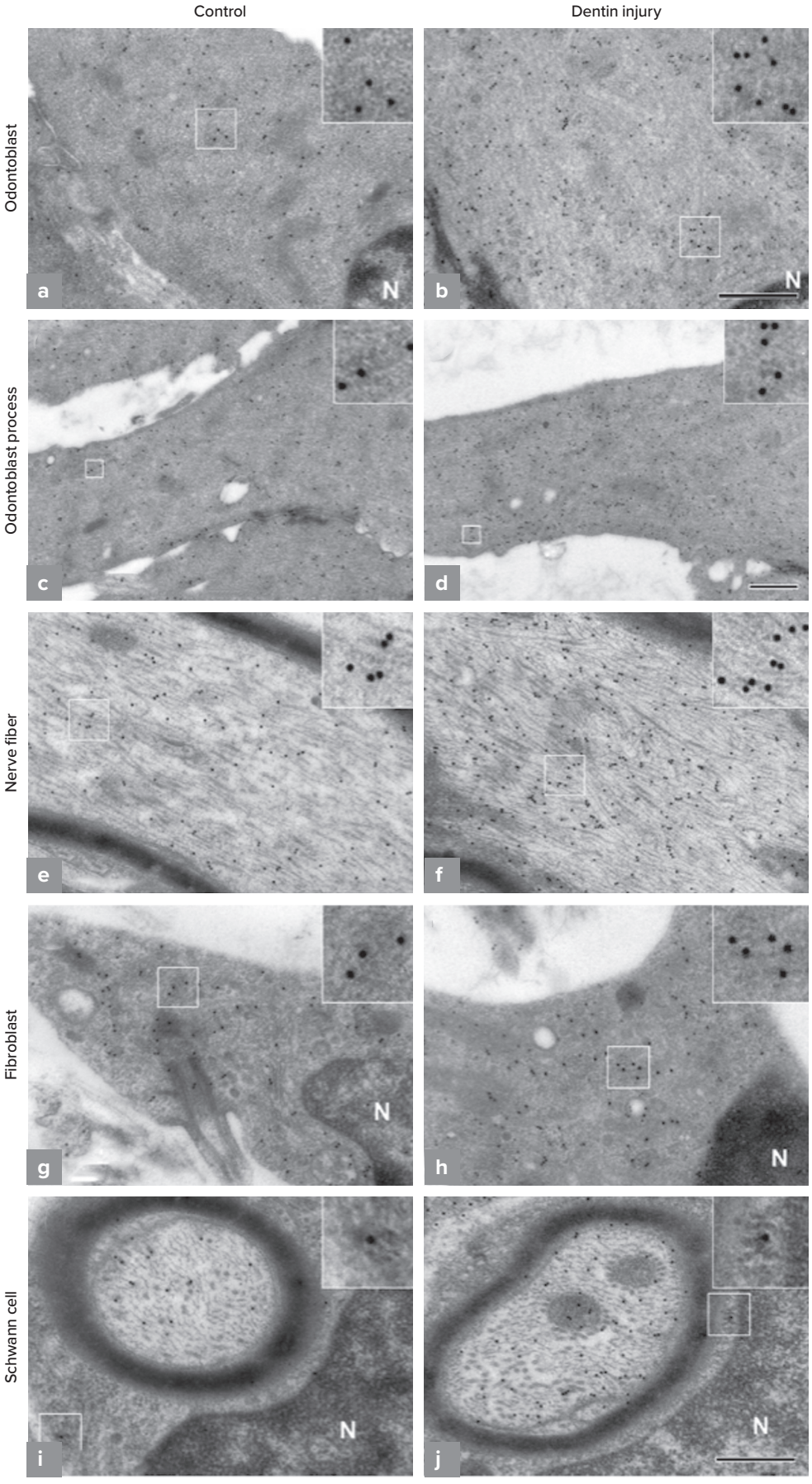


◀ **FIG 4-13** Electron micrographs showing glutamate immunoreactivity in an odontoblast *(a and b)* and its process *(c and d)*, nerve fiber *(e and f)*, fibroblast *(g and h)*, and Schwann cell *(i and j)* in a normal rat dental pulp *(left)* and following dentin injury *(right)*. Gold particles coding for glutamate are dense over the cytoplasm of the odontoblasts and their processes, nerve fibers, and fibroblasts and sparse over the cytoplasm of the Schwann cells and the pulpal extracellular matrix. Insets are enlargements of the boxed areas in the respective figure. N = nucleus; scale bar = 500 nm. (Reprinted with permission from Cho et al.)


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axons that are adjacent to odontoblasts following pulpal inflammation (Fig 4-13) (Cho et al). These findings support the hypothesis that odontoblasts release glutamate when they get activated and then activate GluR expressed on adjacent pulpal nociceptive axons, playing a role in the mechanisms of acute dental pain and hypersensitivity following inflammation (Bae and Yoshida).

Noradrenaline (NA) is a classic postganglionic neurotransmitter present in sympathetic nerves (Lundberg et al 1989). Synthesis of NA requires tyrosine hydroxylase (TH), an enzyme that converts tyrosine to dihydroxyphenylalanine (DOPA), which is then converted to dopamine. Finally, dopamine converts to NA by the enzyme dopamine-β-hydroxylase (DBH). NA acts via adrenergic receptors, which are members of the seven-transmembrane domain G-protein-coupled receptors (Haug and Heyeraas). Stimulation of sympathetic nerves and injection of NA cause vasoconstriction and therefore a fall in PBF (Tönder and Naess, Kim 1985).

Ach is another classical neurotransmitter acting on nerve fibers and blood vessels. It has been associated with parasympathetic fibers, although its presence is not a selective marker for parasympathetic neurons. However, there is evidence for the presence of cholinergic nerves and receptors as well as the Ach-degrading enzyme acetylcholinesterase (AchE) in dental pulp (Avery et al, Olgart 1996). Ach has a vasodilatory effect on blood vessels, and its action seems to be dependent on the presence of nitric oxide (NO) (White et al). Ach is capable of reducing NA release from sympathetic nerves, acting via muscarine receptors located at adrenergic nerve endings. Ach effects are antagonized by atropine (Olgart et al 1996).

## Neuropeptides

Neuropeptides are considered specialized neurotransmitters or neuromodulators. They are synthesized in the cell bodies of neurons, transported to their axons, and released after stimulation, triggering biologic effects by activating receptors located on target cells (Caviedes-Bucheli et al 2008c). Neuropeptides represent the largest class of neurotransmitters/neuromodulators, being up to 50 times larger than classical neurotransmitters (eg, Ach). They are composed of polypeptide chains of 3 to 100 amino acids and are highly conserved through evolution (Holmgren and Jensen). Neuropeptides present multiple target sites for receptor binding compared with smaller classical neurotransmitters (Hokfelt et al).

Neuropeptides are present in every branch of the nervous system in the body, from the central to the peripheral nervous system, including both somatosensory and autonomic neurons (De Swert and Joos). They have several biologic effects, as they may function as neurotransmitters, growth factors, hormones, and immune system signaling molecules, acting in paracrine, endocrine, and neurocrine forms of communication. Therefore, they are the main mechanism by which the nervous system controls the pathophysiology of peripheral inflammation, triggering the so-called "neurogenic inflammation" that takes place in pulpitis and apical periodontitis (Lundy and Linden). Neuropeptides can be divided according to their origin as somatosensory (including substance P [SP], calcitonin gene-related peptide [CGRP], and neurokinin A [NKA]) or autonomic (including neuropeptide Y [NPY] and VIP). Table 4-2 presents a synopsis of neuropeptide functions and effects achieved by binding to their receptors located on target cells.

The first neuropeptide found in the dental pulp (and probably the most researched up to date) was SP (Olgart et al 1977). Several other neuropeptides have been identified in the dental pulp, the most relevant being CGRP, NKA, NPY, and VIP (Casasco et al 1990, Caviedes-Bucheli et al 2006b, Fristad et al 2010). Their origin, expression, distribution, and cellular receptors in teeth and periodontal tissues have been widely studied (Luthman et al 1992, Luthman et al 1988, Caviedes-Bucheli et al 2005a, Caviedes-Bucheli et al 2007b, Caviedes-Bucheli et al 2007c, Caviedes-Bucheli et al 2008b, Caviedes-Bucheli et al 2008c).

Somatosensory neuropeptides are synthesized on ribosomes in the cell body of sensory neurons located at the trigeminal ganglion (Harmar and Keen). Then they are transported via axonal transport both centrally and to the peripheral nerve endings, where they are stored in vesicles waiting to be released upon stimulation (Gulbenkian et al, Price et al). The neuropeptide-containing sensory nerve fibers are located both along blood vessels and as free nerve endings in the tissue. These fibers are also observed at the subodontoblast layer, where they branch toward predentin, with some fibers penetrating 0.1 to 0.2 mm into the dentin as free nerve endings (Wakisaka et al 1984).

SP and NKA are members of the tachykinin family, and both have been found to be present in the dental pulp (Casasco et al 1990, Awawdeh et al, Caviedes-Bucheli et al 2006b). They are released upon various types of noxious stimuli, such as harmful thermal, mechanical, and chemical stimulation of the dentin-pulp complex (Byers et al 1990, Byers and Närhi). Electrical stimulation of unmyelinated afferent C-fibers or the administration of capsaicin has been found to evoke the release of SP (Kemppainen et al). Capsaicin (an extract from red pepper) is a neurotoxic drug that acts on vanilloid receptors expressed on sensory C-fibers; it has been reported to destroy the microtubule system, therefore

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**TABLE 4-2** Origins, receptors, and effects of different neuropeptides

| Neuropeptides | Origin | Cell receptor | Cells expressing the receptor | Vascular effects | Inflammatory effects |
|---|---|---|---|---|---|
| Substance P<br>Neurokinin A<br>CGRP | Somatosensory nervous system (C- and Aδ-fibers) | NK1 (SP)<br>NK2 (NKA)<br>CGRP1/ CGRP2 (CGRP) | • Endothelial cells<br>• Mast cells<br>• Macrophages<br>• Mesenchymal cells<br>• Odontoblasts<br>• Fibroblasts<br>• Lymphocyte T and B cells<br>• NK cells<br>• Dendritic cells | • Blood flow regulation<br>• Vasodilation<br>• Plasma extravasation<br>• Regulation of angiogenic mechanisms by stimulating the release of EGF, FGF, and NO | • Neurogenic inflammation<br>• Immune system stimulator (IL-1, IL-6, IL-12, TNF-α)<br>• Chemotaxis<br>• Enhanced macrophage activity<br>• Increased collagen formation and fibroblast proliferation<br>• Increased lymphocyte proliferation and antibody secretion<br>• Upregulation of matrix metalloproteinases<br>• Mast cell degranulation<br>• Increased NK cell cytotoxicity<br>• Promotion of odontoblast-like cell differentiation<br>• BMP-2 stimulation<br>• Hard tissue formation and tissue reparation |
| Neuropeptide Y | Autonomous nervous system (sympathetic fibers from the superior cervical ganglion) | Y receptor | • Smooth muscle cells<br>• Sensory neurons<br>• Mesenchymal cells<br>• Lymphocyte T and B cells<br>• Dendritic cells<br>• Macrophages<br>• Neutrophils<br>• NK cells | • Blood flow regulation<br>• Vasoconstriction<br>• Regulation of angiogenic mechanisms by promoting the release of VEGF and NO<br>• Proliferation of endothelial cells<br>• Chemotactic for endothelial and smooth muscle cells | • Induction of IL-4, IL-12, TNF-α, and MMP-8 secretion by macrophages<br>• Modulation of adherence and phagocytic capacity of granulocytes and monocytes<br>• Nociceptive and antinociceptive effects<br>• NK cell modulation and $T_h$ cell differentiation modulation<br>• Modulatory role in reparative dentin formation<br>• Neurogenic inflammation inhibition<br>• Anti-apoptosis effects<br>• Proinflammatory cytokine inhibition<br>• Inhibition of SP and CGRP release<br>• Inhibitory role in bone marrow stem cell proliferation and osteoblast differentiation |
| Vasoactive intestinal peptide | Autonomous nervous system (parasympathetic fibers from the otic ganglion) | VPAC1<br>VPAC2 | • Osteoblasts<br>• Osteoclasts<br>• Mast cells<br>• Macrophages<br>• Dendritic cells<br>• Lymphocyte T and B cells | • Blood flow regulation<br>• Vasodilation<br>• Downregulates HIF and VEGF | • Proinflammatory cytokine inhibition (TNF-α, IL-6, IL-12, IL-8)<br>• Promotes IL-10 and TGF-β expression (anti-inflammatory cytokines)<br>• $T_h1$ inhibition and $T_h2$ differentiation (anti-inflammatory effect)<br>• Macrophage inhibition |

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causing a blockage of the afferent and efferent axoplasmic transport (Gamse et al). Moreover, it has been demonstrated that capsaicin infiltration on the inferior alveolar dental nerve provokes a significant reduction in pulpal SP expression (even lower than normal SP basal levels), although an inflammatory process was induced in dental pulp (Caviedes-Bucheli et al 2005b).

NKA is generally coexpressed with SP in the same peripheral nerves. Upon thermal or mechanical stimuli or chemical irritation, inflammatory mediators such as bradykinin and prostaglandins release the two peptides simultaneously (Merighi et al, Grutzner et al). Once released in the tissue, tachykinin neuropeptides mediate sensory and vasoactive functions (McMahon and Koltzenburg, Heyeraas et al 1994) by binding to their specific tachykinin receptors NK1, NK2, and NK3, which show preferences for SP, NKA, and neurokinin B (NKB), respectively (Fristad et al 2003). Neurokinin receptor expression in human pulps has been reported to be significantly greater during clinical inflammatory phenomena (Caviedes-Bucheli et al 2007c).

CGRP-immunoreactive fibers constitute almost half of the dorsal root ganglia neurons (Gibson et al). Immunohistochemical studies have shown CGRP-immunoreactive fibers branching extensively in the coronal pulp near the pulp horns and entering the dentin for up to 0.1 mm (Byers et al 1990). Most of the CGRP-positive sensory neurons exhibit slow conduction velocities (typical of unmyelinated C- and small myelinated Aδ-fibers); only 10% to 15% of CGRP-positive neurons have faster conduction velocities in the range of the myelinated Aβ-fibers (Alvarez et al). There are two known types of CGRP receptors: CGRP1 and CGRP2. Calcitonin receptor–like receptor (CRLR) is an important component of the CGRP1 receptor complex. CRLR mRNA has been found in human dental pulp and trigeminal neurons by reverse transcriptase–polymerase chain reaction (RT-PCR) (Edvinsson et al). CGRP receptor expression in human pulps has also been reported to be significantly greater during pulp inflammation (Caviedes-Bucheli et al 2005a).

NPY (sympathetic) and VIP (parasympathetic) are the principal neuropeptides derived from the autonomic nervous system, and both have been identified in the dental pulp (Uddman et al 1980, Uddman et al 1984, Luthman et al 1992, Rodd and Boissonade 2002, Caviedes-Bucheli et al 2006b). NPY is synthesized in sympathetic neurons originating in the superior cervical ganglion and supplied to nerve terminals by axonal transport (Fried et al 1985); it modulates numerous physiologic processes including appetite, blood pressure, and circadian rhythms (Wahlestedt and Reis). NPY fibers accompany blood vessels and characteristically encircle them, reaching the subodontoblastic plexus, while some end as free nerves in the odontoblastic layer, particularly at the pulp horns (El Karim et al 2006b). NPY is co-stored with NA; they release together on sympathetic nerve activation by stress, physical exercise, myocardial ischemia, and life-threatening situations (Pernow, Chronwall and Zukowska). Six human NPY receptor subtypes have been identified, named Y1 to Y6. NPY Y1 receptor has been found in the human pulp tissue by means of RT-PCR, using specific primers to detect mRNA for NPY Y1 (Uddman et al 1998).

VIP is expressed in dental pulp neurons and is suggested to be of parasympathetic origin (Uddman et al 1980, Caviedes-Bucheli et al 2006b). VIP fibers travel along small and large blood vessels showing a network-like arrangement adjacent to the blood vessel walls; however, very few VIP fibers reach the subodontoblastic layer (Wakisaka et al 1987). Contrary to other neuropeptides, it seems that noxious stimulation of dental pulp does not induce VIP release, even during an inflammatory process (Caviedes-Bucheli et al 2006b), creating controversy on the precise stimuli that trigger VIP release from parasympathetic neurons. NO is one possible culprit because it has been shown to induce VIP release from enteric ganglia and submandibular glands (Grider and Jin, Modin et al). VIP receptors are called VIP/pituitary adenylate cyclase–activating polypeptide receptor (VPAC)-1 and VPAC2 (previously VIPR1 and VIPR2) (Harmar et al). VPAC1 in pulp tissue provides a functional role of VIP in pulpal health and disease (El Karim et al 2006a).VPAC receptors are expressed in osteoclasts/osteoblasts (Lundberg et al 2000a) and in other immune cells such as macrophages, mast cells, and B and T lymphocytes (Sakakibara et al), suggesting that VIP plays an important role as a modulator of immune response (Fig 4-14) (Ganea and Delgado, Caviedes-Bucheli et al 2007b).

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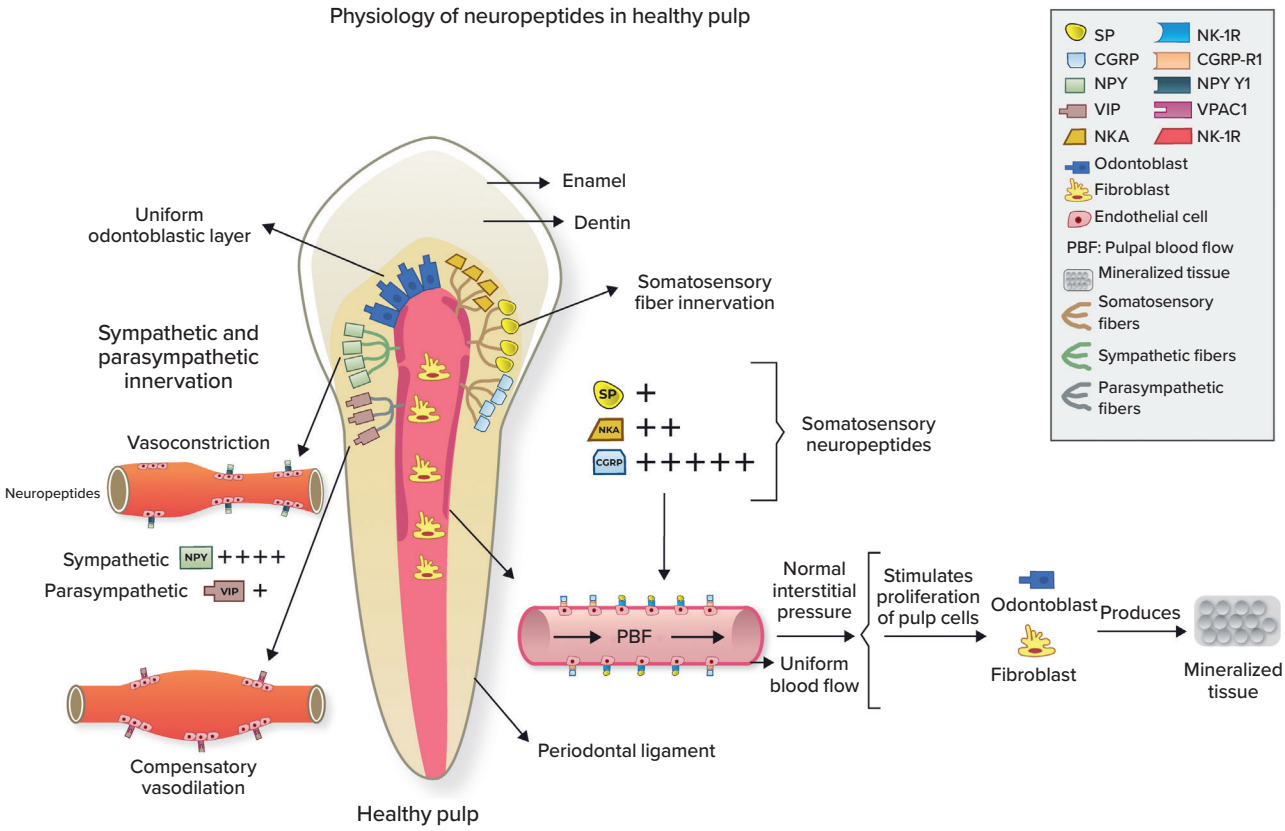


▲ **FIG 4-14** The nervous system controls the physiology of the pulp tissue through the expression of neuropeptides. Somatosensory fibers express proinflammatory neuropeptides such as CGRP (the most abundant +++++), NKA (++), and SP (the least expressed but most potent vasodilator +). Sympathetic and parasympathetic fibers also express anti-inflammatory neuropeptides such as NPY (the second most abundant ++++) and VIP (+). NPY induces vasoconstriction while VIP induces compensatory vasodilation as a mechanism for regulating PBF. This interaction is necessary for the stimulation and proliferation of fibroblasts, odontoblasts, and pulp cells responsible for the formation of mineralized tissue as a defense mechanism.

## Pain Mechanisms of the Dentin-Pulp Complex

The three major steps in the perception of odontogenic pain are detection, processing, and perception. Detection is a primary function of peripheral nociceptive neurons (eg, Aδ- and C-fibers), which get sensitized or depolarized when stimulated by noxious physical stimuli or inflammatory mediators that act on receptors located in terminal endings. Although these peripheral nociceptors appear to be simple free nerve endings, they are biochemically specialized and express several different receptors and ion channels that provide them with the ability to detect noxious thermal, chemical, and mechanical stimuli (Henry and Hargreaves). There are several types of receptors located in the nerve endings that could start the detection process (Fig 4-15), including G-protein–coupled receptors (Schöneberg et al), voltage-gated ion channels (Yu et al 2005), sodium channels (voltage-gated Na-v) (Lai et al), potassium channels (voltage-gated K-v) (Ocaña et al), calcium channels (voltage-gated Ca-v) (Catterall et al), transient receptor potential channels (TRPs) (Clapham et al 2005), capsaicin receptors (Vanilloid receptors) (Szolcsanyi), and neurotrophin receptors (TRK) (Kobayashi et al). Besides triggering the local release of neuropeptides, these "polymodal detectors" generate action potentials that transmit sensory information to the central nervous system. Therefore, these receptors constitute key therapeutic targets due to their importance in pain signaling, as they are involved in the initiation and propagation of action potentials and the neurotransmitter release necessary for synaptic transmission (Henry and Hargreaves).


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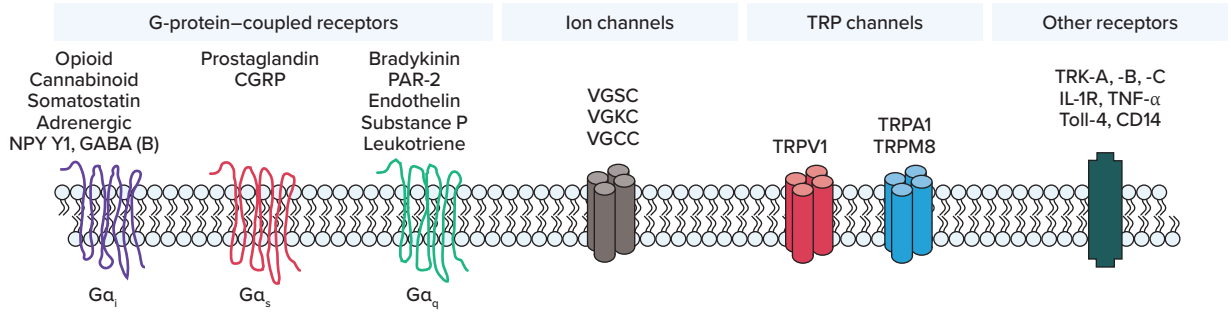


▲ **FIG 4-15** Schematic representation of the major classes of receptor or ion channels present on peripheral terminals of sensory neurons that serve to transduce external stimuli into altered neuronal function. Not all receptors or ion channels are present on all neurons, and several have been shown to be altered during inflammation or nerve injury. PAR-2 = protease-activated receptor subtype 2; TRPA1 = transient receptor potential A1; TRPM8 = transient receptor potential M8; TRPV1 = transient receptor potential V1 (the capsaicin receptor); VGCC = voltage-gated calcium channel; VGKC = voltage-gated potassium channel; VGSC = voltage-gated sodium channel. (Based on Henry and Hargreaves.)

Sodium channels have been suggested to play a role in increased neuronal excitability as well as in spontaneous ectopic activity triggered by inflammatory or neuropathic pain. It has been shown that, under acute inflammation, the expression and function of various isoforms are altered, particularly the tetrodotoxin-resistant isoforms, due to prostaglandin signaling (Priest et al). This provides an explanation as to why drugs that act on sodium channels (ie, local anesthetics) may be inefficient under this scenario (Reisman et al); therefore, dispensing an ibuprofen premedication could help by reducing the amount of prostaglandins, allowing sodium channels to function normally (Gould et al). Capsaicin receptor (TRPV1) is also relevant under inflammatory conditions, as it is responsible for mediating primary and secondary hyperalgesia (Davis et al). It has been found in peptidergic and nonpeptidergic fibers in human pulp (Renton et al). This receptor is sensitized by inflammatory mediators, including bradykinin, prostaglandins, and serotonin, among others, and therefore plays an important role in neuropathic pain mechanisms (Caterina and Julius).

In an inflamed dental pulp, nociceptive afferent fibers get activated by the presence of inflammatory mediators, such as cytokines, prostaglandins, histamine, bradykinin, serotonin, NO, hydrogen ions, and nerve growth factor (NGF). The activation of nociceptors leads to neuropeptide release, which contributes to the sensitization of nerve fibers that will start to display spontaneous depolarization or reduced threshold for depolarization and increased response to normal and noxious stimuli (Chung et al).

Peripheral afferent sensory neurons also respond to neurotrophins such as NGF, increasing the synthesis of SP and CGRP and the sprouting of terminal fibers. Sprouting causes an increase in the density of innervation of the inflamed tissue, contributing to increased pain sensitivity in chronic pulpal inflammation (Byers and Närhi). NGF has also been shown to sensitize TRPV1, generating hyperalgesia (Dyck et al, Bonnington and McNaughton).

After activation, Aδ- and C-fibers transmit nociceptive signals to the trigeminal nuclear complex, up to the outer layer of the medullary dorsal horn, where the processing of pain takes place (Tandon et al). At this location, sensory fibers transmit information by releasing glutamate and neuropeptides, SP, or CGRP. These molecules bind to their receptors located on postsynaptic projection neurons that transmit the nociceptive signal to the thalamus (Sessle). From the thalamus, another neuron sends this information to the cerebral cortex, where pain perception finally occurs.

Under certain inflammatory conditions, Aβ-fibers (normally innocuous mechanoreceptors) may express SP and develop new central connections in the dorsal spinal cord, which can cause an extreme mechanical allodynia. One clinical example of this scenario is the patient suffering with an acute apical abscess, where even a gentle touch of the affected tooth evokes pain. In addition to these peripheral sensitization mechanisms, central sensitization might also occur, caused by a barrage of impulses from C nociceptors, leading to the central release of glutamate and SP, thereby activating the central receptor for both molecules. In this condition, stimulation of the Aβ-fibers would generate a pain response, constituting a central mechanism of allodynia (Neumann et al).

## Neural Interactions with Microvasculature and Control of PBF

The close relationship of nerve fibers with the pulp microvasculature has been widely demonstrated in

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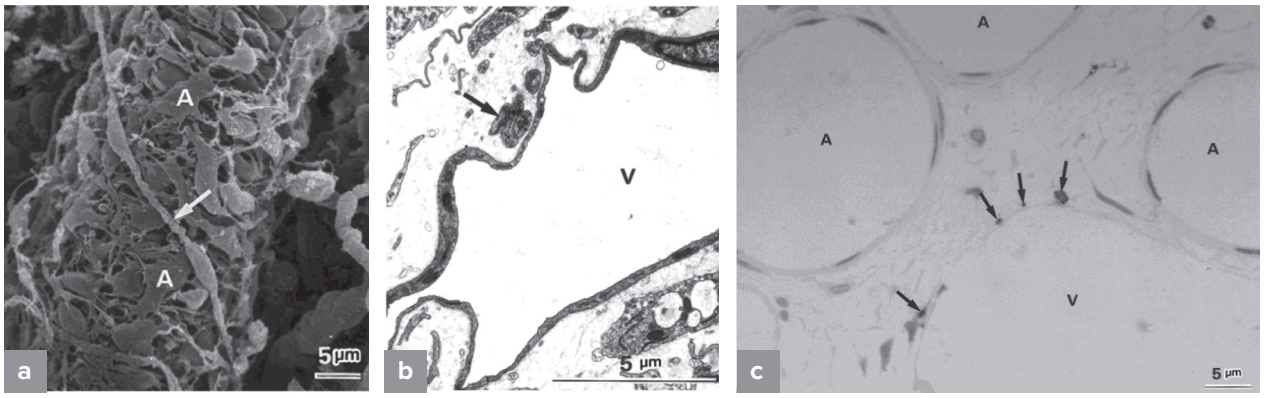


▲ **FIG 4-16** Examples of the close relationship between nerve fibers and blood vessels. *(a)* Scanning electron micrograph showing tunica adventitia of an arteriole with a nerve bundle *(arrow)* running along the adventitial cells (A), with which fine cytoplasmic processes interconnect to form a rough meshwork. *(b and c)* Micrographs showing perivascular nerves of microvessels in rat dental pulp. Note that a postcapillary venule (V) is densely supplied by CGRP-R1 nerve fibers *(arrows* in *c)* and nerves containing large granular vesicles *(arrow* in *B).* (Reprinted with permission from Iijima and Zhang.)

immunohistochemical observations (Fig 4-16). Most of the vasomotor nerves are adrenergic, because the arterioles are the most densely innervated vessels. Therefore, the role of neuropeptides in regulating blood flow and plasma extravasation has been studied over the years (Wakisaka and Akai, Okamura et al, Zhang et al 1998). Hemodynamic regulation in the dental pulp is essential to provide optimal nutrition to pulpal cells and for the removal of metabolic products from the tissue, maintaining a pulpal blood pressure in harmony with pulpal tissue pressure (Olgart 1996).

NPY and NA get released simultaneously from sympathetic nerve terminals (Ekblad et al), stimulating vasoconstriction in the dental pulp and oral mucosa and thereby reducing the blood flow (Kim et al 1989). These effects are mainly mediated by α-adrenergic receptors located on the vascular smooth muscle cells of arterioles and venules (Kim et al 1984) located primarily in the pulp horns near the odontoblastic region. At the apical region, the sympathetic control is less evident (Okamura et al).

Sympathetic control of blood flow also has an influence on pain signaling, as it has been shown that the excitability of intradental sensory nerves is inhibited when sympathetic vasoconstriction occurs (Edwall and Scott). This could explain why patients may suffer fluctuations in pulpal pain intensity when visiting the dentist due to stress-induced vasoconstriction (Olgart 1996). However, in chronic inflammation, the increased sympathetic activity and release of NA might directly excite nociceptors due to the presence of α-adrenergic receptors on sensory nerve endings (Sato et al). On the other hand, a stimulus such as deep cavity preparation and thermal irritation may eliminate the sympathetic vasoconstriction for several hours (Forssell-Ahlber and Edwall). This is also observed in the presence of histamine, bradykinin, SP, and Ach (Edwall et al 1973). In addition, NPY has an angiogenic effect, showing that sympathetic nerves are important during tissue development and repair (Zukowska-Grojec et al).

Autonomic innervation is known to be responsible for short-term control of blood pressure by baroreceptors that depend mainly on the innervation of the heart and blood vessels (Smith et al, Suojanen et al). This control mechanism could have some effects on autonomic neuronal control of pulpal perfusion, as alteration in human body position has been shown to cause a significant change in PBF (Firestone et al, Ajcharanukul et al). Normally, PBF is maximized during sleep in a resting supine position, mainly due to minimal sympathetic vasoconstrictor activity in the pulp, but when the pulp is inflamed, further increase of already-raised pulp tissue pressure will occur (Heyeraas and Kvinnsland, Heyeraas and Berggreen). This increase is sufficient to stimulate intradental nerves, giving rise to insomnia due to a severely throbbing toothache. If the individual stands up again, the sympathetic innervation of the pulp will lower perfusion pressure, allowing tissue pressures to fall somewhat and thereby relieving pulpal pain (Ajcharanukul et al).

Many of the functions of the sympathetic neurons are opposed by activation of parasympathetic neurons. Therefore, VIP induces vasodilation in the cat pulp when systemically infused (Olgart et al 1989). The vascular effect of VIP is dependent on NO, as it has been observed that


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VIP vasodilation is reduced after administration of NO synthase inhibitor (Modin et al).

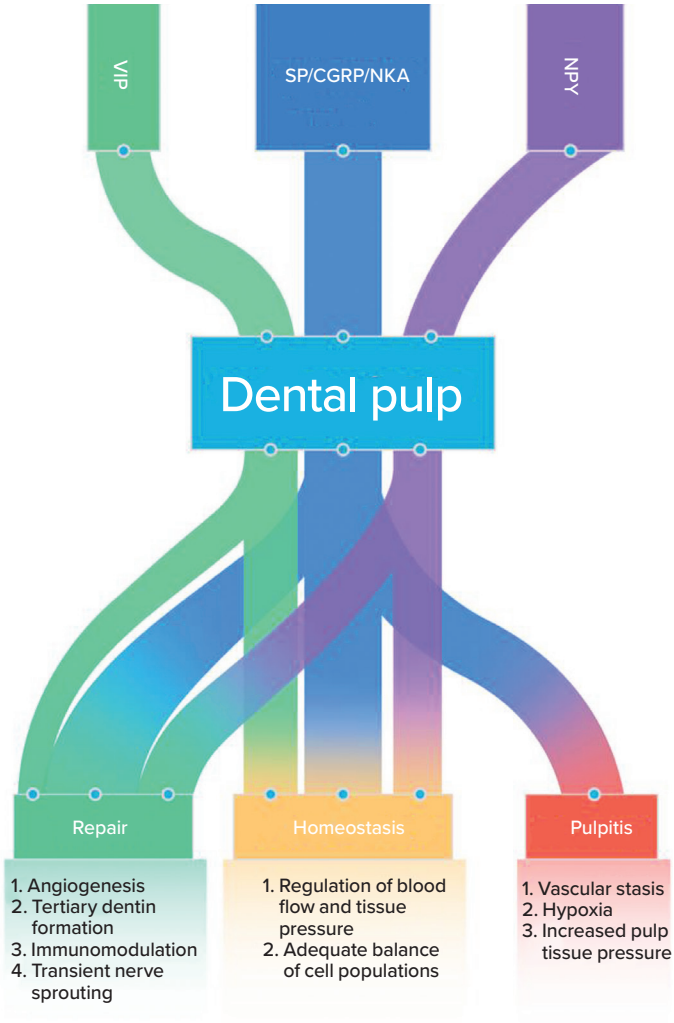
The control of PBF from adrenergic nerves is more relevant when tissue is irritated. SP and CGRP have potent vasodilator effects in dental pulp vessels, while NKA has a much smaller effect on PBF (Gazelius et al 1987). Somatosensory neuropeptides are released upon activation of nociceptors, either by direct stimulation on the tooth crown (ie, drilling or probing of exposed dentin) or by brief antidromic electrical stimulation of the inferior alveolar nerve, inducing an increased vascular permeability in the pulp with a long-lasting blood flow increase (Gazelius and Olgart, Matthews and Vongsavan). The initial response of the vasodilator effect is mediated by SP, while the continued long-lasting rise in blood flow is more dependent on CGRP (Caviedes-Bucheli et al 2008c). Figure 4-17 summarizes the main actions of neuropeptides in homeostasis control, as well as in the generation of pulpitis and in repair mechanisms.

## Neural Responses to Tooth Injuries

The dental pulp undergoes physiologic changes due to various normal stimuli (eg, chewing) as well as when reacting to pathologic situations such as caries (El Karim et al 2006b) or chemical aggressions during dental restorative procedures, including the use of local anesthetics (Caviedes-Bucheli et al 2009). The placement of adhesives, cement, or whitening materials (Caviedes-Bucheli et al 2008a, Caviedes-Bucheli et al 2010, Caviedes-Bucheli et al 2013) can also produce damage to the dental pulp. Mechanical traumatic injuries have also been documented as pulpal irritants, including cavity preparations (Caviedes-Bucheli et al 2005c), orthodontic forces (Caviedes-Bucheli et al 2011b), and occlusal trauma (Caviedes-Bucheli et al 2011a, Caviedes-Bucheli et al 2021).

### Neural reactions to caries

The main disease that affects the human dental pulp is dental caries. The cariogenic microorganisms and their metabolic products produce inflammatory and immunologic reactions that affect the dentin-pulp complex (El Karim et al 2006b). The dental pulp is capable of mounting an innate immune response to caries, which slows bacterial invasion. Inflammation due to caries leads to the activation of adaptive immunity, which in turn leads to an increase in the inflammatory process and intrapulpal edema (Hahn and Liewehr 2007a, Hahn and Liewehr 2007b).




▲ **FIG 4-17** Neuropeptides maintain homeostasis via regulation of vasodilatory and vasoconstrictive mechanisms in healthy dental pulp. Therefore, an adequate concentration of all neuropeptides regulates pulpal blood pressure, pulpal oxygenation levels, and acceptable levels of immunocytes for immunosurveillance *(orange box)*. SP, CGRP, and NKA are released from C-fibers *(blue)*, which are the most important fibers in the dental pulp tissue and therefore depicted with a thick line. Under mild stress, the concentration of all these neuropeptides increases via a transient nerve sprouting (neuroplasticity). C-fibers also govern the repair response *(green box)* and are depicted with a thick line *(blue)*. NPY- and VIP-releasing fibers are depicted with medium and thin lines because of their relative importance in dental pulp physiology and repair regulation. Through their ubiquitous receptors, neuropeptides activate different populations of immunocytes (inflammatory and anti-inflammatory), angiogenic mechanisms (to increase oxygenation levels), and tertiary dentin formation. If the injury disappears, the nerve sprouting also disappears; however, if the injury persists, less prevalent nerve fibers such as VIP- and NPY-releasing fibers become necrotic (*red box* only connects with C-fibers). This creates a neuromodulatory imbalance in which a vasodilatory and proinflammatory response dominates. The imbalance adds to the already stressed tissue and generates vascular stasis, increased pulpal tissue pressure, and hypoxia *(red box)*. Clinically, these anatomical and physiologic changes result in pulpitis, which might lead to pulpal necrosis.

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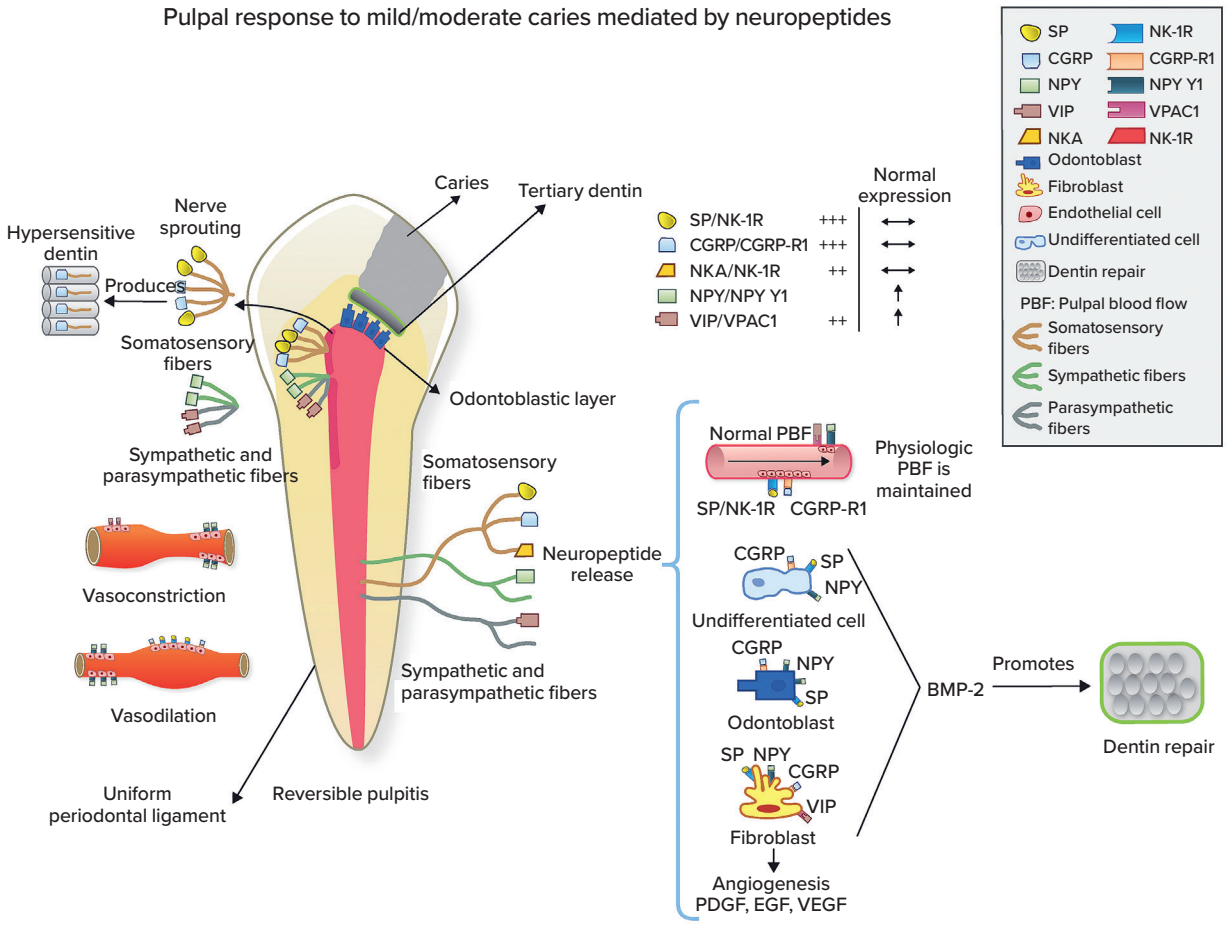


▲ **FIG 4-18** The pulpal response to mild/moderate caries begins with the sprouting of sympathetic and somatosensory fibers in the dentin near the caries site, clinically producing dentinal hypersensitivity. Neuropeptides such as SP, CGRP, NKA, and NPY are released, inducing a reversible inflammatory reaction with vascular changes of vasoconstriction and vasodilation. PBF is not affected due to activation of the parasympathetic system, and VIP is released as a compensatory mechanism of vasodilation for control of the vascular tone of the pulp. SP, CGRP, and NPY stimulate endothelial cells, fibroblasts, and inflammatory cells to induce angiogenesis, a mechanism by which hypoxic areas in the pulpal tissue are controlled. These neuropeptides, through their NK1, CGRP1, and NPY Y1 receptors, bind to odontoblasts and fibroblasts to induce them to produce reparative dentin as a defense mechanism.

In a relatively slowly progressing caries lesion, where the damage is considered mild or moderate, the pulpal response is usually reversible (capable of healing), and tertiary reactive dentin is formed (Fig 4-18). In rapidly progressing caries lesions, where there is severe damage to the pulp, reactive dentin is no longer produced, and a reparative atubular dentin is formed instead, with increased cellular and molecular interactions (Bjorndal), generating a neurogenic inflammatory response as a defense mechanism, where the nervous system controls the vascular and immune systems through the release and actions of neuropeptides (Caviedes-Bucheli et al 2008c). If the condition is not treated, necrosis of the dental pulp will occur (Fig 4-19).

Somatosensory neuropeptides such as SP, NKA, and CGRP and their NK1, NK2, and CGRP-R1 receptors are increased in pulps of carious teeth compared to healthy pulps. CGRP exerts many regulatory functions in pulp physiology, such as maintaining vascular tone, ensuring a uniform blood flow and a constant supply of nutrients to the tissue, regulating interstitial pulp pressure, and stimulating the growth of pulp cells, such as fibroblasts and odontoblast-like cells (Awawdeh et al, Rodd and Boissonade 2000, Caviedes-Bucheli et al 2005a, Caviedes-Bucheli et al 2006b). Unlike these neuropeptides, autonomic NPY (and its NPY Y1 receptor) and VIP (and its VIP-C1 receptor) are expressed proportionally to caries status, being higher in mild and moderate caries than in severe processes, indicating a clear regulatory role in the early stages of the disease (Gibbs and Hargreaves, El Karim et al 2006a, El Karim et al 2008).

Cellular and neurovascular interactions are crucial for the pulpal response to dental caries, generating


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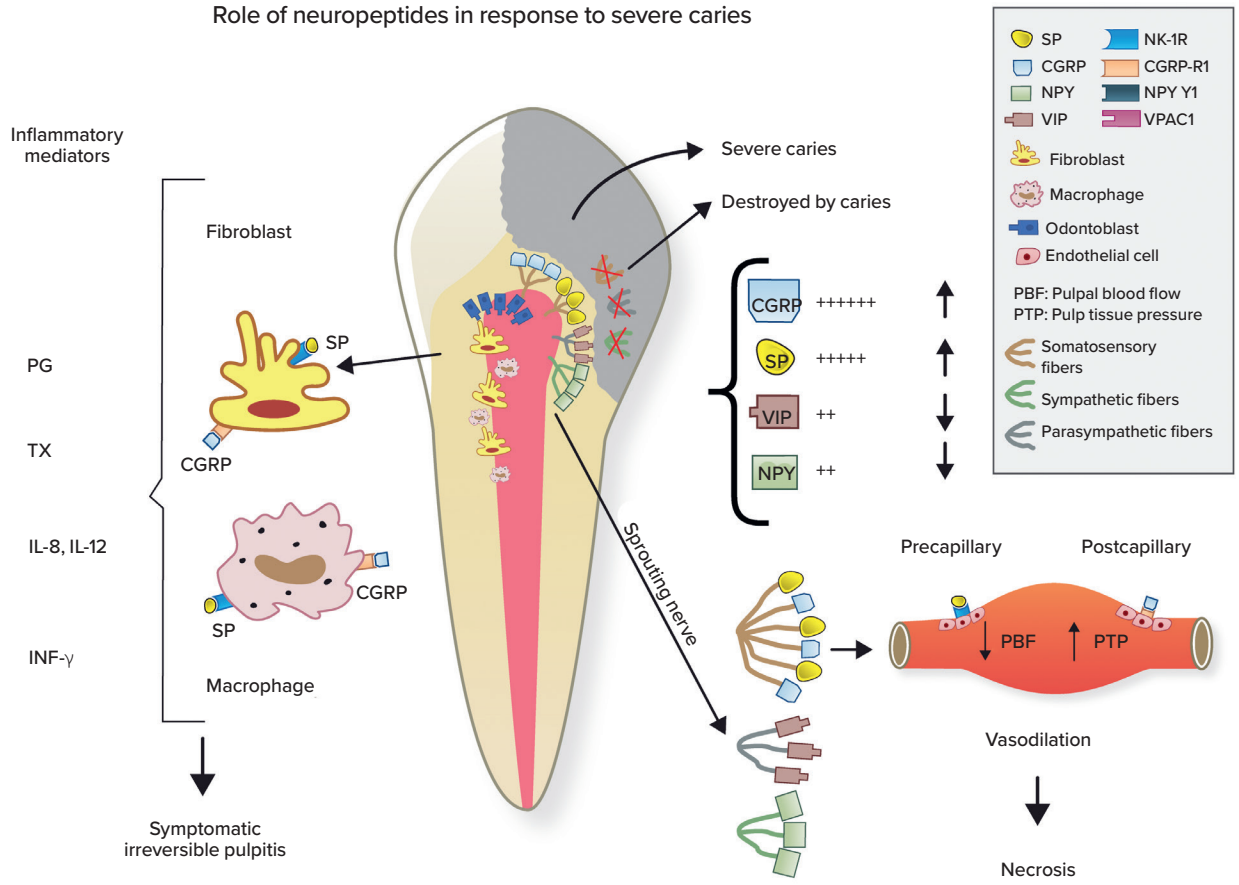


▲ **FIG 4-19** When severe caries is present, there is an initial sprouting of somatosensory, sympathetic, and parasympathetic fibers in the pulp tissue adjacent to the caries lesion area, with a large increase in the expression of somatosensory neuropeptides such as SP and CGRP at double their basal values. Neuropeptide release generates an irreversible inflammation process, characterized by great vasodilation, decreased PBF, increased pulp tissue pressure (PTP), and accumulation of inflammatory mediators such as thromboxane (TX), prostaglandins (PG), interleukins (IL-8, IL-12), and interferon-γ (INF-γ) induced by fibroblasts and inflammatory cells such as macrophages. Clinically, these reactions lead to stages such as acute irreversible pulpitis or pulp necrosis. The sympathetic and parasympathetic fibers that sprout in the dentin are destroyed by the carious process, evidenced by a decrease in their expression, avoiding the possibility of inflammatory control generated by somatosensory neuropeptides.

vasodilation and increased vascular permeability in addition to recruitment and activation of immune cells and dental pain control mechanisms. Vasoactive neuropeptides (SP, CGRP, and NKA) are released in the presence of cariogenic bacteria through an axon reflex that is directly proportional to the level of bacterial aggression and the stage of caries development (Rodd and Boissonade 2000, Lundy and Linden).

SP is the least expressed neuropeptide in healthy pulps, but its expression is significantly increased in caries-provoked painful pulpitis than in asymptomatic teeth with large caries lesions. This increased SP expression may be an effort to regulate PBF by controlling interstitial fluid exudate related to the inflammatory process (Rodd and Boissonade 2000, Awawdeh et al, Caviedes-Bucheli et al 2006b). On the other hand, CGRP is the most expressed neuropeptide and the main mediator of arterial vasodilation in the dental pulp, potentiating the action of SP and NKA and therefore resulting in a transient increase in interstitial pressure and PBF (Heyeraas 1985). This is reflected in the movement of the dentinal fluid, generating pain after any thermal, mechanical, or chemical stimulus as a defense mechanism (Heyeraas 1989). Finally, NKA expression presents a very similar trend to that of SP, since both are tachykinins coexisting in the same nerve fibers. However, the higher expression of NKA could be explained by the fact that this neuropeptide can also be expressed in nonneuronal cells, such as human endothelial cells and different types of inflammatory and immune cells (Caviedes-Bucheli et al 2006b).

During the early stages of mild and moderate caries, the interstitial fluid from the transient neurogenic

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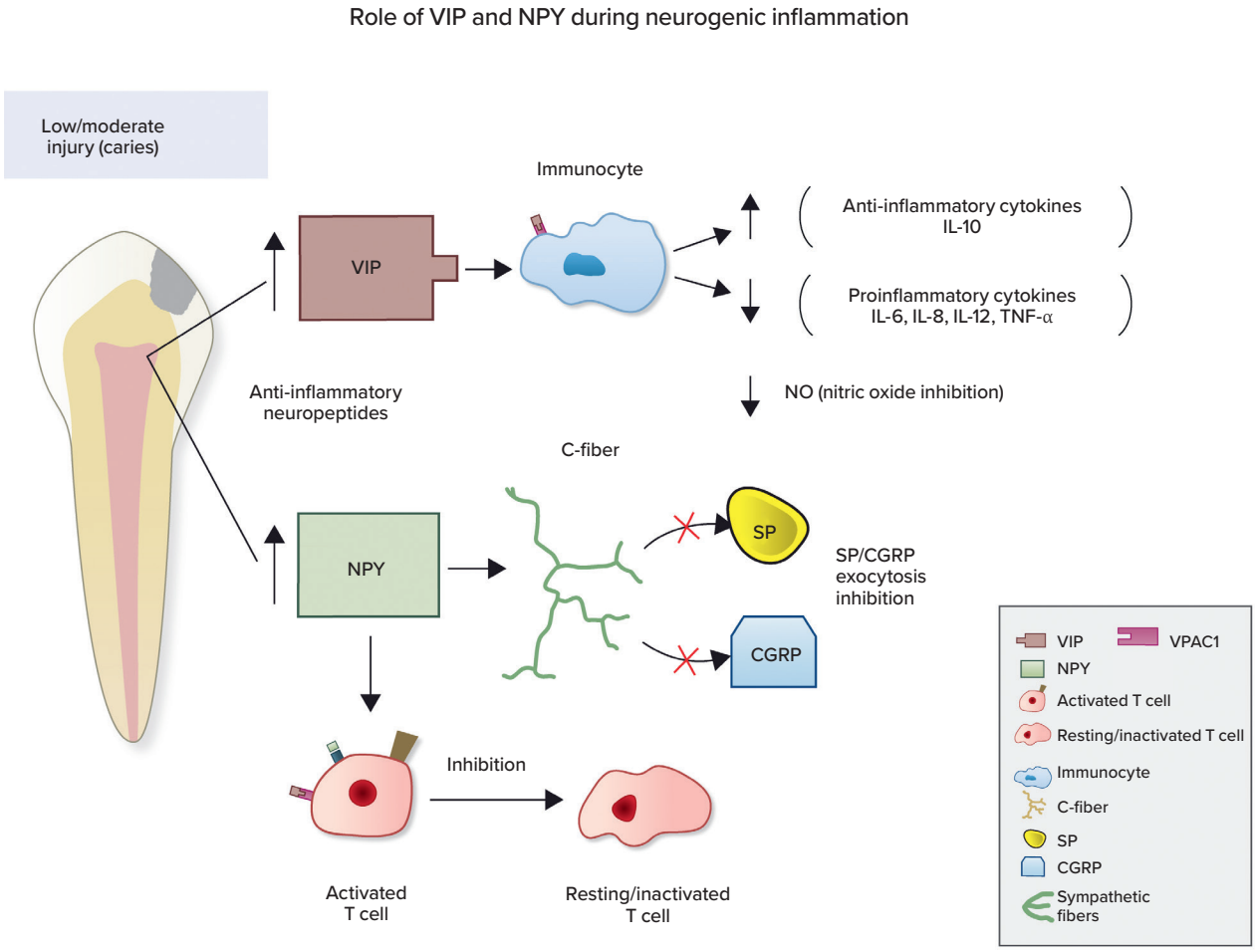


▲ **FIG 4-20** VIP and NPY mainly play an inhibitory role during neurogenic inflammation and counterbalance the proinflammatory effects of SP and CGRP. In low- to moderate-intensity injuries, parasympathetic fibers release VIP, which, upon interaction with its receptor (VPAC1) located in various immunocytes, stimulates the expression of anti-inflammatory cytokines such as IL-10 and inhibits proinflammatory mediators such as NO, IL-6, IL-8, IL-12, and TNF-α. On the other hand, the sympathetic neuropeptide NPY inhibits SP and CGRP exocytosis from C-fibers, inactivates T cells, and modulates the $T_h1/T_h2$ response.

inflammation is absorbed into the circulatory and lymphatic systems through edema-prevention mechanisms, and there may be normal basal levels of neuropeptides. In an advanced cariogenic process, if the pulp is not capable of resolving the increased tissue pressure and the elevated levels of these neuropeptides, a persistent edema takes place, contributing to pain and local necrosis (Heyeraas 1985, Heyeraas 1989). The intratubular location of somatosensory and sympathetic nerve fibers and their participation in the outward movement of dentinal fluid during neurogenic inflammation indicate that the neural component is important in the initial vascular response to dental caries (Vongsavan and Matthews).

Sympathetic innervation also participates in the neural response to the carious process. It has been demonstrated that NPY and its receptor NPY Y1 exhibit increased expression in carious teeth than in noncarious teeth; within carious teeth, the level of NPY is much higher in cases of mild and moderate caries than in severe caries. Therefore, this neuropeptide plays a role in modulating pain, the immune response, pulpal inflammation, angiogenesis, and the formation of reparative dentin during mild or moderate cariogenic stages (Fig 4-20). However, in a severe carious process, necrotic tissue and the destruction of sympathetic fibers impede this control (El Karim et al 2008). Although parasympathetic innervation does not seem to have an important role in the regulation of PBF like the sympathetic nervous system (Olgart 1996), VIP expression is slightly increased in mild and moderate stages of dental caries with respect to the normal pulps (El Karim et al 2006a). This may be a control mechanism to regulate the vasoconstriction produced by the sympathetic system through NPY (Fig 4-21) (Caviedes-Bucheli et al 2008c).


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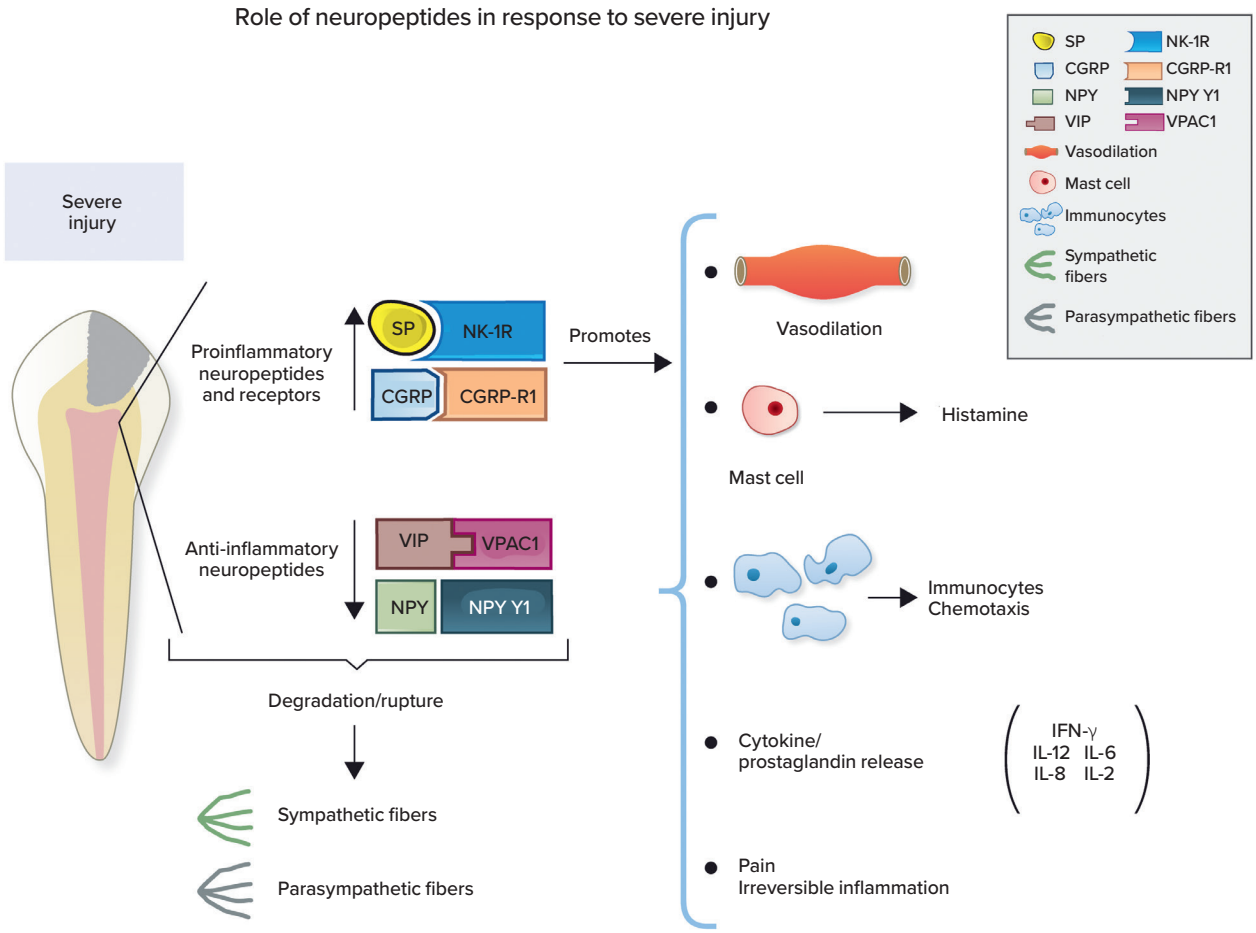


▲ **FIG 4-21** In a severe injury, VIP/NPY-releasing nerve fibers become necrotic, and vasoactive neuropeptides like SP, CGRP, and NKA govern the neurogenic inflammation. They activate various immune cells, inducing the release of proinflammatory cytokines and promoting vasodilation, which, if sustained over a long period of time, could lead to pulp necrosis due to an increase in pulp tissue pressure and hypoxia.

In carious teeth, NPY is expressed mainly in free nerve endings in the dental pulp and nerves that project toward the odontoblastic layer and predentin (where they are not associated with blood vessels), suggesting that in mild or moderate caries, it can participate in the formation of reparative dentin, which may occur due to the expression of the NPY Y1 receptor in pulp fibroblasts (Killough et al 2010). The biologic effect of NPY on fibroblasts promotes tertiary dentin formation by increasing their expression of bone morphogenetic protein 2 (BMP-2), which stimulates the secretory activity of odontoblasts. Moreover, NPY also promotes the migration and proliferation of endothelial cells in addition to stimulating the formation of capillary tubules, generating an angiogenic process that is crucial for healing and reparative dentin formation (El Karim et al 2009). Angiogenesis is a natural defense mechanism of the dental pulp against any type of aggression, including caries, where the fibroblast is the predominant cell, as it is responsible for the synthesis and maintenance of the extracellular matrix, providing a structural framework for the tissue (Fig 4-22). Therefore, fibroblasts play an important role in pulp healing as a subpopulation that has the ability to differentiate into dentin-forming cells (Hargreaves et al 2003a).

VIP also participates in the repair process of mild and moderate caries; it acts as an angiogenic inducer by having receptors in pulpal fibroblasts (El Karim et al 2006a), inducing the expression of various angiogenic factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF) (El Karim et al 2009). Angiogenic factors are essential for the formation of reparative dentin (Caviedes-Bucheli et al 2017, Gomez-Sosa et al 2019) as a defense mechanism in mild or moderate carious processes. In

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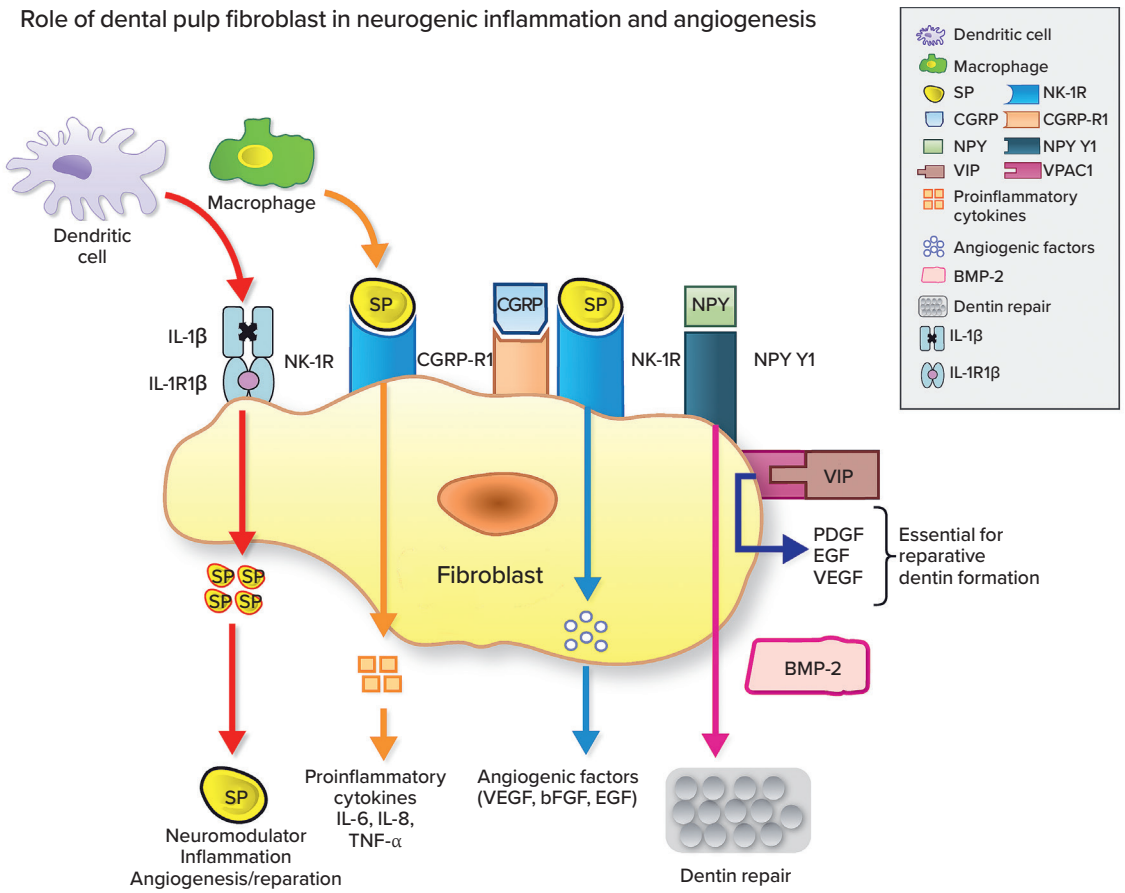


▲ **FIG 4-22** Dental pulp fibroblasts (DPFs) play an essential role during pulpal inflammation. Via IL-1β secretion, dendritic cells stimulate SP release by fibroblasts, a proinflammatory neuropeptide that governs the immune and vascular response. Besides regulating M1/M2 macrophage differentiation, fibroblasts also express NK1 and CGRP-1 receptors. Upon secretion of SP by M1 macrophages, this neuropeptide interacts with its receptor. This interaction triggers a cascade of events that stimulate the release of various proinflammatory cytokines such as IL-6 and IL-8. Both CGRP and SP interact with DPFs in order to activate the release of several angiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF,) and epidermal growth factor (EGF). DPFs also express NPY Y1 and VIP receptors and are capable of interacting with these anti-inflammatory neuropeptides. NPY stimulates the release of BMP-2 by DPFs, which in turn stimulates odontoblasts to secrete reparative dentin. The role of this cell in reparative dentin processes is further evidenced by the release of platelet-derived growth factor (PDGF), EGF, and VEGF when VIP interacts with its receptor located on DPF membranes.

severe or rapidly progressing caries, large vascular changes and immune reactions take place, potentiating neurogenic inflammation (Rodd and Boissonade 2000).

The extremely rich innervation of the dental pulp also generates an immune response, directly stimulating immunocompetent cells through neuropeptides and facilitating the chemotaxis and accumulation of immune cells in the pulp tissue (Hahn and Liewehr 2007a). When the pulp is inflamed due to severe caries, SP, CGRP, and NKA are highly expressed, generating a cellular and immune response with the release of inflammatory mediators such as histamine from mast cells and chemotaxis of macrophages and phagocytes for the release of cytokines, prostaglandins, and thromboxanes (Caviedes-Bucheli et al 2008c). SP is a chemoattractant for T cells and increases antigen- and mitogen-induced production of interleukin 2 (IL-2) and interferon gamma (IFN-γ). It also increases IL-12 production by antigen-presenting cells and IL-8 production by pulp cells, both of which are proinflammatory substances released as a defense mechanism against the aggression of dental caries bacteria (see Fig 4-21) (Park et al).

NPY and VIP expression is significantly increased in mild and moderate caries, controlling the immune response in the dental pulp (Rodd and Boissonade 2002). Moreover, CGRP


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and VIP recruit immature dendritic cells to sites of acute inflammation and inhibit migration of mature dendritic cells to regional lymph nodes, which helps to phagocytose dental caries aggressors (Hahn and Liewehr 2007b).

Fibroblasts also express SP and the NK1 receptor as a defense against the carious process, where their levels are increased with respect to those of the normal pulp, responding to several proinflammatory cytokines, such as IL-1β, IL-6, and IL-8, which are induced by SP and tumor necrosis factor α (TNF-α). The effect of proinflammatory cytokines suggests that fibroblasts play a neuromodulatory role in the response to caries and any type of dental injury (see Fig 4-22) (Killough et al 2009). Moreover, these inflammatory cytokines and chemokines can induce hyperalgesia, probably mediated by prostanoids and NGF release or by NPY from sympathetic fiber activation (Jonakait and Hart).

NPY modulates neurogenic inflammation by inhibiting SP release from sensory neurons. This limits neurogenic inflammation when NPY is increased in mild and moderate caries (El Karim et al 2008). It has been shown that endogenous sympathetic neurotransmitters inhibit exocytosis of SP and CGRP in afferent pulp peptidergic nerves, generating inhibition of the inflammatory process and antinociception by the NPY Y1 receptor (Hargreaves et al 2003a). The increase in the NPY Y1 receptor expression suggests a suppressive action on pulpal inflammation due to dental caries. At the immune level, this NPY Y1 molecule inhibits the response of T cells, meaning that NPY and its receptor are necessary for the proper functioning of antigen-presenting cells and at the same time can protect against infection and excessive inflammation by inhibiting the hyperactivity of T cells and other pulp cells such as fibroblasts (see Fig 4-20) (El Karim et al 2008).

Pulpal fibroblasts are also considered a target cell for NPY, modulating pulpal inflammation, pain, and healing by releasing and reacting to several proinflammatory cytokines (El Karim et al 2006b). Therefore, the fibroblast has a neuromodulatory role in the response to caries, being essential for bidirectional communication between inflammatory cells and nerve fibers in the pulpal response to caries (see Fig 4-22) (Killough et al 2010).

VIP also modulates the response of the pulp to dental caries at the immunologic level, because it is a powerful endogenous anti-inflammatory agent that can regulate the inflammatory process in mild caries. Its expression has been demonstrated in immune and inflammatory cells by modulating an immune response through generalized inhibition of proinflammatory cytokines, NO, and upregulation of anti-inflammatory cytokines such as IL-10, via the VIP receptor in mild and moderate caries (see Fig 4-20) (Rodd and Boissonade 2002, Lundy and Linden).

In addition, the immune cells of the pulp secrete other neuropeptides such as somatostatin and β-endorphins to achieve homeostasis of neurogenic inflammation, which are more numerous in inflamed pulp (Mudie and Holland). Somatostatin is a nonvasoactive neuropeptide that inhibits the antidromic release of SP (Gazelius et al 1981). Somatostatin and CGRP suppress T cell proliferation and their IL-2 and IFN-γ release (Lambrecht). Endorphins are opioid peptides that cancel the effects of vasoactive neuropeptides, controlling pain and inflammation in the initial stages of dental caries (Cabot et al).

## Neural reactions to local anesthetics and vasoconstrictors

The use of anesthetic solutions is essential for pain control during dental clinical procedures. They act by reversibly blocking neural transmission via binding to sodium channels on nerve fibers (White and Durieux). The more common local anesthetics used in dentistry are those of the amide type, such as lidocaine and mepivacaine, which have a rapid onset and a medium duration of anesthetic effect. Prilocaine is considered a secondary amide that has a rapid onset but shorter duration of anesthetic effect. There are also other classes of amide anesthetics, such as articaine and bupivacaine, that have a rapid onset and long-lasting anesthetic effect (Ogle and Mahjoubi).

In addition to inhibiting sensory nerve activity, local anesthetics also reduce vascular tone, which generates an increase in blood flow at the injection site; this leads to a rapid elimination of the anesthetic solution and therefore decreases the duration of anesthetic effect. In consequence, they are usually applied in conjunction with a vasoconstrictor to prolong the anesthetic effect (Chng et al). Vasoconstrictors act on the α-adrenergic receptors in the blood vessels, causing a decrease in tissue blood flow that prevents the anesthetic solution from being rapidly absorbed into the systemic circulation, thereby providing a longer duration of anesthetic effect (Odor et al).

Adrenaline (epinephrine) is the most powerful vasoconstrictor used in dentistry. It is used in combination with anesthetic solutions such as lidocaine and mepivacaine at a concentration of 1:80,000 or 1:100,000. When combined with prilocaine, the concentration is reduced to 1:200,000 (Pitt Ford et al). The use of adrenaline in local anesthetics in dentistry has been shown to reduce PBF by decreasing the diameter of arterioles and venules when administered by infiltrative techniques (Yu et al 2002).

Felypressin (octapressin) is another vasoconstrictor used in combination with some local anesthetic solutions. It acts directly on vascular smooth muscle cells. The usual concentration when used with 3% prilocaine is 0.03 IU/mL. This vasoconstrictor does not reduce blood flow as much

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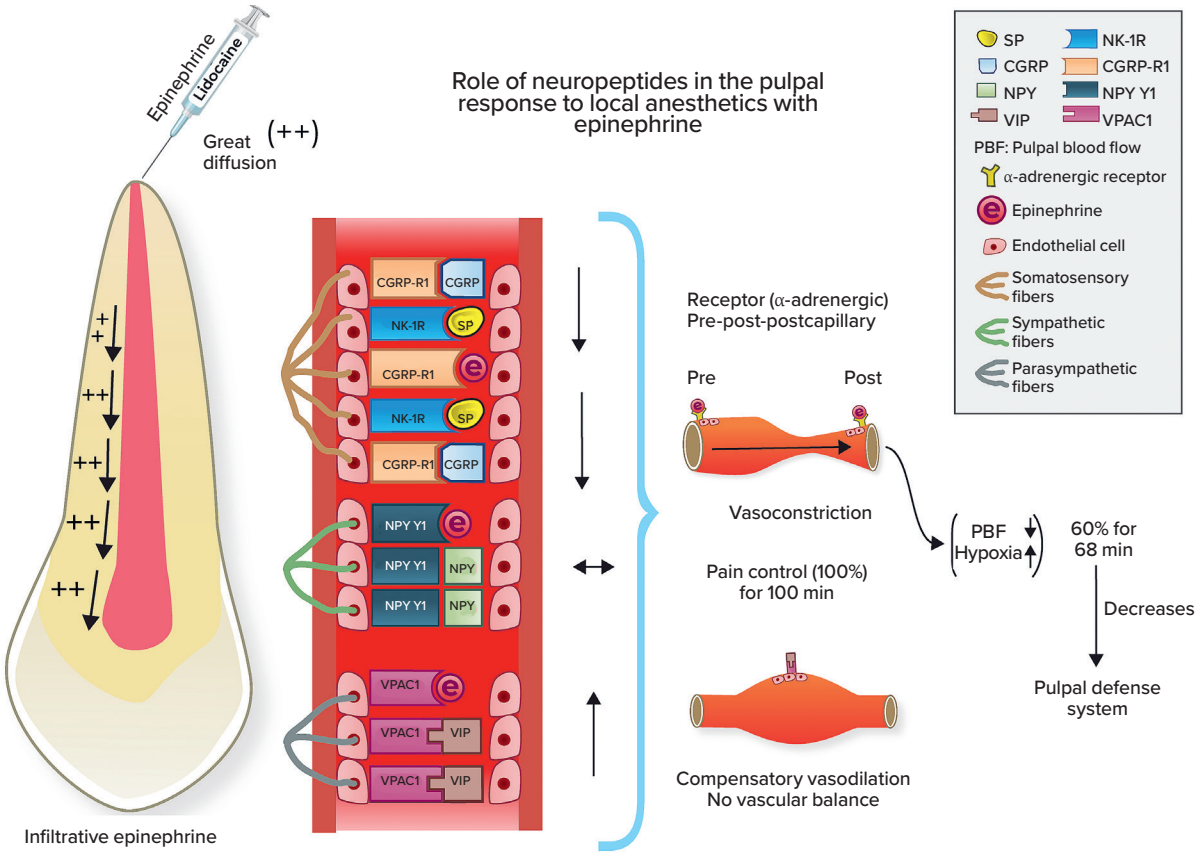


▲ **FIG 4-23** The most commonly used local anesthetic in dentistry is lidocaine with epinephrine as a vasoconstrictor. When applied via infiltration, it controls pain completely (by 100%) for 100 minutes. At the vascular level, it generates a decrease in PBF by 60% for 68 minutes, making the pulp vulnerable to the aggression of dental procedures due to the great diffusion of epinephrine in the pulp tissue as a polypeptide with low molecular weight. At the level of the nervous system, it attenuates the expression of SP and CGRP, reducing their physiologic action at the vascular level by competing (with epinephrine) for the pre- and postcapillary α-adrenergic receptors and probably the CGRP-R1 of the blood vessels, generating areas of hypoxia that decrease defense capacity of the pulp tissue.

adrenaline and therefore gives the anesthetic solution a shorter duration of anesthetic effect. If the concentration of felypressin is increased, the duration of anesthetic effect decreases. This differs from adrenaline, where the effectiveness increases with concentration (Olgart and Gazelius).

Lidocaine is a powerful vasodilator, so it requires a powerful vasoconstrictor such as adrenaline to compensate for this antagonistic effect. Prilocaine has no vasodilatory action, so its use with a weak vasoconstrictor such as felypressin is feasible (Kim 1986, Ahn and Pogrel 1998, Yu et al 2002). When 2% lidocaine without vasoconstrictor is used with an infiltrative technique, the duration of the anesthetic effect is 25 minutes, and it does not have any significant effect on PBF, making it adequate for pain control for a short period of time (Chng et al). However, it has been found that 1 minute after infiltration injection of 2% lidocaine with 1:100,000 adrenaline, PBF drops to 60%, and 6 minutes later it drops further to 28%, with an inhibition of sensory nerve activity of 100 minutes. This is four times the duration of 2% lidocaine anesthesia without vasoconstrictor (Olgart and Gazelius, Chng et al). PBF may take up to 75 minutes to restore, and consequently during this period the dental pulp may experience inadequate oxygenation and become vulnerable to aggressive dental clinical procedures (Fig 4-23) (Kim et al 1984). However, the infiltrative use of 3% prilocaine with felypressin 0.03 IU/mL has no inhibitory effects on PBF and provides good control of sensory nerve activity, so it is considered a good option when an infiltrative technique is needed before performing a dental procedure (Fig 4-24) (Tachibana et al).


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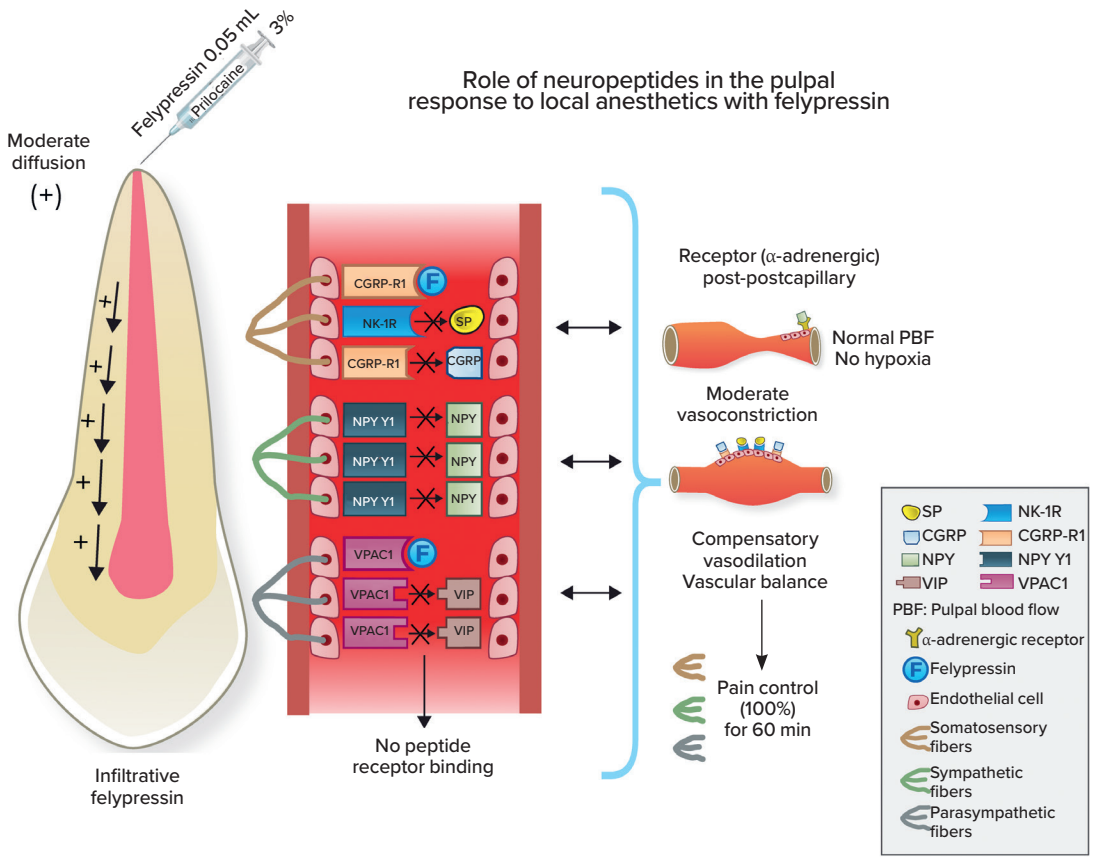


▲ **FIG 4-24** Infiltration of prilocaine with felypressin as a vasoconstrictor controls pain completely (by 100%) for 60 minutes without producing alterations in the pulp microcirculation. Felypressin has moderate diffusion in the dental pulp because it is a larger polypeptide than epinephrine and has a vasoconstrictor effect acting only on α-adrenergic receptors at postcapillaries of the blood vessels of the dental pulp. The expression of SP, CGRP, NPY, and VIP remains stable, controlling the homeostasis of the pulpal tissue and maintaining normal PBF without generating areas of hypoxia, thus preserving the defense capacity of the dental pulp against the aggression of dental procedures.

The vasoconstrictor effect of adrenaline (epinephrine) and felypressin on PBF differs due to their action mechanism. Adrenaline acts on α-adrenergic receptors at the precapillary and postcapillary levels, providing a potent vasoconstrictor effect (Olgart and Gazelius), while felypressin acts only on postcapillary α-adrenergic receptors, generating a moderate vasoconstrictor effect (Ahn and Pogrel 1998). This difference in action mechanism can be explained by the diffusion limits of these vasoconstrictors. Adrenaline is a small polypeptide that can very easily reach the blood vessels of the dental pulp when the anesthetic is placed via infiltration, whereas felypressin is a larger polypeptide and its diffusion levels do not reach the blood vessels of the pulp when anesthesia is applied with an infiltrative technique (Olgart and Gazelius). When anesthesia is applied with a nerve block distant from the tooth (ie, inferior alveolar nerve block), the effect of vasoconstrictors is not significant on PBF (Pertl et al).

Vasoconstrictors used in anesthetic solutions induce dynamic changes that regulate blood flow and inflammatory responses to noxious stimuli on the pulp tissue (Byers and Närhi). These changes are highly influenced by nerve fibers, including unmyelinated C-fibers and some Aδ-fibers that are in close association with blood vessels (Nair, Olgart 1996). SP and CGRP have an important role in maintaining tissue homeostasis by regulating PBF at a basal level according to tissue needs (Hargreaves et al 1994). The more aggressive the local anesthetic, the more implications it will have in the prognosis of pulpal health, which is closely related to the control of PBF and the blockage of sensory nerve activity. Several studies have shown that 2% lidocaine and 4% prilocaine, when used without

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a vasoconstrictor with an infiltrative technique, preserve basal levels of SP and CGRP, maintaining tissue homeostasis of the pulp by maintaining a vasodilator tone at rest (Berggreen and Heyeraas, Caviedes-Bucheli et al 2009).

When an anesthetic solution such as 2% lidocaine or 2% prilocaine without vasoconstrictor is applied, the vasodilator effect of vasoactive neuropeptides such as SP and CGRP attenuates the vasoconstrictor effect of sympathetic fibers and NPY, maintaining hemodynamics of the pulp tissue (Lin et al). When an adrenaline-type vasoconstrictor or felypressin is used, on the other hand, the effect of NPY is potentiated, drastically reducing PBF and generating a state of vulnerability of the pulp to dental procedures. Therefore, VIP with its vasodilator effect plays an important role in the regulation of PBF (Caviedes-Bucheli et al 2008c, Caviedes-Bucheli et al 2009). These previously described clinical situations can be explained at the inflammatory level in that adrenaline-type vasoconstrictors decrease the expression of SP and CGRP in the pulp tissue, inhibiting their vasodilator action (Pertl et al), generating a decrease in blood flow, altering the supply of nutrients to the cells and components of the pulp, and delaying the elimination of toxins and metabolic waste products, thereby causing an accumulation of potentially inflammatory substances that can generate an irreversible injury when the dentin-pulp complex is damaged by dental clinical procedures (Kim 1985, Ahn and Pogrel 1998).

It has been shown that C-fibers also contain α- and β-adrenergic receptors where vasoconstrictors could act and consequently reduce or attenuate the release of SP and CGRP, because SP coexists with CGRP within the same type of nerve fiber that generates vascular smooth muscle contraction and reduced blood flow (Yu et al 2002, Hargreaves et al 2003b). When SP expression was measured in groups of pulps infiltrated with anesthetic with or without vasoconstrictor, SP levels were significantly lower in the pulps of teeth in which no vasoconstrictor was used (Caviedes-Bucheli et al 2009). The reduction in SP levels could be attributed to the inhibition action of vasoconstrictors on the α-adrenergic receptors located in the nociceptors of the pulpal nerve fibers, which can reduce the release of this neuropeptide (Hargreaves et al 2003b). Furthermore, lidocaine with adrenaline competes with SP for the same NK1 receptor, which could result in a reduction of the neuropeptide-receptor interaction; consequently, SP-dependent pulpal defense mechanisms may be affected (Li et al 1995).

This could have clinical significance in terms of hemodynamic changes that must take place when the dentin-pulp complex is exposed to dental procedures (Fristad). It has been shown that SP has a mitotic effect on fibroblasts, endothelial cells, and smooth muscle cells of the blood vessels of the dental pulp (Trantor et al), as well as an effect on the recruitment of immunocompetent cells, thus playing an important role in the healing and repair of the pulp (Bowles et al). Prilocaine 3% with felypressin 1:200,000 also generates a significant decrease in SP levels; however, tissue hypoxia caused by felypressin is less critical due to the minor reduction in blood flow as compared to adrenaline (Tachibana et al, Ahn and Pogrel 1998).

It could be recommended that 4% prilocaine without vasoconstrictor and 3% prilocaine with felypressin should be used by infiltrative injection in vital teeth undergoing dental procedures. When infiltrative anesthesia with vasoconstrictor is needed, felypressin would be the ideal vasoconstrictor. Adrenaline can result in irreversible alterations of pulpal functions when used with 2% lidocaine with infiltrative techniques. When mandibular or conductive anesthesia is needed, the effect of vasoconstrictors is not significant on PBF (Caviedes-Bucheli et al 2009).

## Neural reactions to cavity preparations

The pulpal neural response to restorative dentistry depends on several factors, including thermal, chemical, and mechanical irritation; the thickness of the remaining dentin; and the biocompatibility of the dental materials (About et al). The effects of cavity preparation on the pulpal tissue depend on other factors such as the design of the bur used, the speed of rotation and torque, the amount of force applied to the bur, the cutting time, and the cooling efficiency of the irrigation (Lee et al 1992, Hatton et al). A healthy pulp is able to defend itself, and most effects of restorative procedures on the pulp are minor and transient (Murray et al).

Sensory nerve reactions to restorative procedures can be classified as immediate, early, and late. For each of these stages, the neural response depends on the severity of the pulpal injury and the stages of inflammation and healing (Byers et al 1992). Immediate responses take place during the first few minutes and include destruction of nerve fibers in the injured dentin and pulp, hypersensitivity of surviving fibers, and a neurogenic inflammatory phenomenon from the release of neuropeptides in the dental pulp (Caviedes-Bucheli et al 2008c). Early responses occur during the first few days after cavity preparation and are characterized by nerve fibers sprouting into the surviving pulp, increasing axonal transport and neuropeptide content. Sensory fibers containing CGRP greatly outnumber those containing SP and grow toward the surviving odontoblasts and the pulpal tissue surrounding the lesion (Caviedes-Bucheli et al 2004, Caviedes-Bucheli et al 2005c). Delayed responses occur over the following weeks; nerve fibers accompany the repair tissue as it gradually replaces the acute inflammation. Nerve sprouting decreases when inflammation is reduced, and


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reparative dentin eventually covers the injury site (Byers et al 1992).

Studies of inflamed pulps after cavity preparation in human teeth have shown immediate reactions of sensory nerve sprouting in the adjacent pulp within the first 2 days, with the release of neuropeptides such as SP and CGRP, suggesting that the budding intensity can be correlated with dental pain following cavity preparation (eg, dentinal hypersensitivity) (Ferrari and Byers). It has also been suggested that the growth of sensory fibers is due to the presence of the trophic factor NGF, whose expression is increased during trauma such as cavity preparation. NGF is also capable of stimulating the growth of nerve fibers. NGF synthesis is increased by pulp fibroblasts located near the lesion, which shows the importance of these cells in repair mechanisms. Once the release of sensory neuropeptides is triggered, a cascade of events takes place that ends with the reduction of inflammation and the formation of reparative dentin. Contrary to what happens with the sensory fiber network, the NPY-IR sympathetic nerves do not show increased sprouting after cavity preparation with pulp exposure. Due to this difference, it has been suggested that either NGF does not govern the pulpal sympathetic system or that the response to this molecule is different (Dalsgaard et al, Byers 1992, Haug et al). However, there is also evidence that an increase in sprouting of sympathetic fibers occurs *during* cavity preparations, for which NGF is believed to play a vital role. It is believed that these sympathetic fiber sprouts may play an important role in the formation of reparative dentin by stimulating odontoblasts (Shimeno et al).

Under normal conditions, the network of NPY-IR sympathetic fibers is less numerous and dense than the network of sensory fibers. Despite this morphologic difference, the evidence suggests that both networks are capable of interacting with each other, since the activation of sympathetic fibers can inhibit the release of neuropeptides by sensory fibers, affecting the reparative processes orchestrated by the somatosensory system. Also, the network of sensory fibers increases its density by performing selective sympathetic denervation and vice versa. In cavity preparations, it has been shown that the sympathetic system exerts some type of influence on the phenomenon of neurogenic pulpal inflammation, as a greater number of mast cells are found when there is an imbalance of sympathetic fibers. This suggests that during processes of severe injury to the dental pulp, necrosis of the sympathetic fibers could trigger events that perpetuate or worsen the inflammatory process by generating more pain in the teeth, due in part to a greater availability of NGF (Haug et al).

CGRP causes an inflammatory reaction, including increased blood flow and permeability of blood vessels in the damaged dental pulp. In addition, the blood flow reaction induces differentiation of the immune system on peripheral tissues, facilitating hard tissue formation and inducing tissue repair from an early stage (Takamori). During deep cavity preparation without pulp exposure, SP shows significantly higher expression in the pulps shortly after cavity preparation compared to baseline levels. These basal SP levels show a large variation among patients, providing a potential explanation as to why some people are more susceptible to pain and swelling after a procedure, such as cavity preparation, and therefore tend to require root canal therapy more than others (Caviedes-Bucheli et al 2005c).

In summary, neural response to cavity preparation is directly related to the levels of aggression carried out on the dental pulp during this procedure (Fig 4-25). The increase in temperature in the pulp can generate a severe inflammatory phenomenon of a neurogenic nature that occurs, for example, when using a dull bur, which requires the application of greater pressure to cut, leading to an increase of temperature due to friction in the tooth, and thereby generating damage to the pulp tissue (Thomas and Kundabala). When the temperature of the pulp increases above 109.4°F/43°C, irreversible damage occurs. There is an alteration of the pulpal microcirculation that leads to dilation of the blood vessels in an environment of low tolerance to temperature (Kodonas et al). Because the pulp is surrounded by hard tissues (ie, enamel, dentin, and cementum), an accumulation of inflammatory mediators occurs, followed by obstruction of capillaries and finally thrombosis of entire sections of the pulp. The response is mediated more by sensory afferent innervation of the pulp than sympathetic innervation (Raab).

This response can be aggravated by the use of local anesthetics with vasoconstrictor when they are injected by infiltration techniques, because they alter the thermoregulation of the pulp. An increase in temperature from 98.6°F/37°C to 108.5°F/42.5°C results in an increase in blood flow by 100%, on average, and plasma extravasation as a defense mechanism. The vasoconstrictor produces a reversible blockade of this protective mechanism against the harmful temperatures generated by the cavity preparation, which seems to be considerably reduced when infiltrative anesthesia is used (Sukapattee et al). The dental literature has shown a 3% to 25% rate of pulpal necrosis due to tooth preparation for full-crown restorations (Byers et al 1992). The vitality of the pulp was reduced in a manner directly proportional to the thickness of the remaining dentin; that is, the deeper the cavity, the more inflammation and the greater the possibility of necrosis of the pulp, which influences more than just the nature of the dental materials that are placed on the preparation cavity (Wisithphrom et al).

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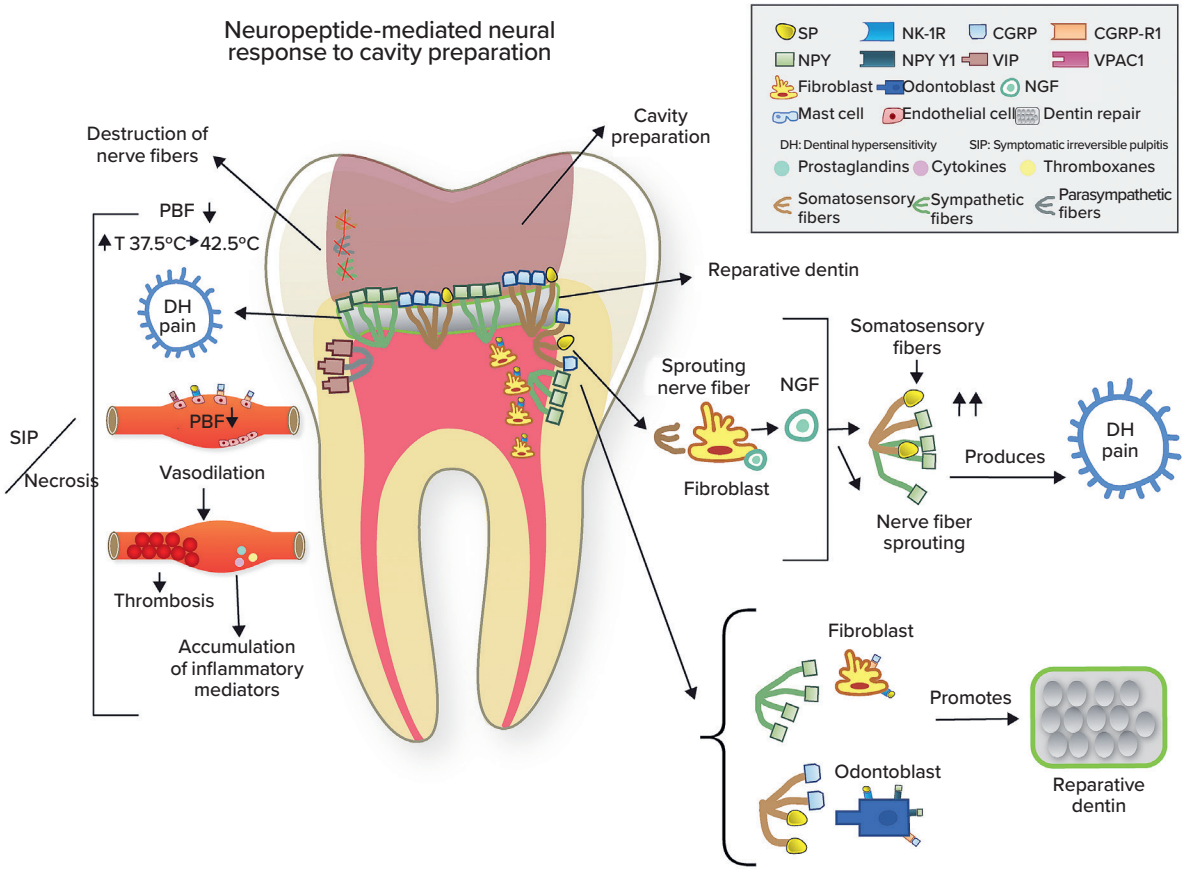


▲ **FIG 4-25** The immediate response of dental pulp to cavity preparation when the cutting temperature is controlled at 37°C includes destruction of nerve fibers in affected dentin and in injured pulp, with hypersensitivity of the remaining fibers. The early response occurs days later, where there is sprouting of somatosensory fibers, increasing the content of SP and CGRP. The sprouts grow toward the surviving odontoblasts and fibroblasts, and the sympathetic fibers remain stable, expressing NPY and clinically generating pain such as dentinal hypersensitivity (DH). In late stages, the growth of sensory fibers is due to the presence of NGF, whose synthesis is increased by fibroblasts in response to cavity trauma, which promotes the formation of reparative dentin as a defense mechanism. When the cutting temperature is not controlled and exceeds 42.5°C, a 100% increase in PBF will occur, with an increase in interstitial pressure, generating thrombi and accumulation of inflammatory mediators that can lead to a clinical stage of irreversible pulpitis or necrosis.

## Neural reactions to dentin bonding and cementation agents

### Bonding agents

Continuous development of dental products has provided a wide range of biomaterials to address various clinical conditions in dentistry. However, despite these advances, there is still a need for a biomaterial that meets all the requirements to be used as a bonding agent, including antimicrobial effect, high biocompatibility, and good mechanical properties (Costa et al 2003).

Direct restorations rely on bonding agents to enamel and dentin that are photopolymerized compounds (Bouillaguet). They are generally classified as self-etching or one-step bonding agents and total-etching bonding agents (Ribeiro et al). Most of these adhesive systems are composed of methacrylate monomers such as hydroxyethyl methacrylate (HEMA), which is converted into a solid compound through the polymerization process. However, only 50% to 75% of the monomers polymerize, while the rest remain as free radicals, mainly reactive oxygen species (ROS), which can diffuse through dentinal tubules (Costa et al 1999). These agents constitute a biologic risk for the pulp because there is a link between ROS production and cytotoxic activity (Atsumi et al, Spagnuolo et al).

These events could generate enough damage to induce apoptosis because the pulp cells, especially the fibroblasts, are exposed to oxidative stress, damaging lipids, proteins, and DNA (Spagnuolo et al 2004, Demirci et al), thus reducing cell viability and mitochondrial activity


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(Costa et al 2000). ROS produces an inflammatory infiltrate of mononuclear cells and multinucleated giant cells in dental pulp tissue (Jontell et al). They also affect the pulpal microcirculation and could trigger a neurogenic inflammatory reaction whereby neuropeptides such as SP, CGRP, NPY, and VIP are released (Caviedes-Bucheli et al 2008c). SP is released as a defense response of the pulp to caries. Its concentration increases with cavity preparation, and it reacts with dentin bonding agents when used in direct restorations on the pulp tissue, creating an inflammatory process with a cumulative effect (Caviedes-Bucheli et al 2005c, Caviedes-Bucheli et al 2010).

The remaining dentin thickness over the pulp plays an important role after cavity preparation. Residual thickness greater than 0.5 mm has a protective effect on the dental pulp against external factors, such as dentin bonding agents (Pashley, Hatton et al al). Bonding agents can induce a mild or moderate neurogenic inflammatory reaction, which releases neuropeptides such as SP, CGRP, and NPY (Caviedes-Bucheli et al 2010). When the thickness of the remaining dentin is less than 0.5 mm, the presence of monomers has been observed in the odontoblastic layer and in the predentin zone (Tay et al), inducing a foreign body reaction characterized by the presence of an inflammatory infiltrate of macrophages and multinucleated giant cells (Gwinnett and Tay). Moreover, monomers affect mitochondrial activity in fibroblasts and the secretion of inflammatory mediators in macrophages (Jontell et al); they also inhibit the proliferation of T lymphocytes and interrupt the cell cycle of pulp cells by inducing apoptosis (Mantellini et al). This generates a significant inflammatory reaction with a large release of somatosensory neuropeptides such as SP and CGRP (Grutzner et al, Caviedes-Bucheli et al 2010).

Bonding agents require the previous application of a conditioner in order to perform their functions on the dental structure. These conditioners generally are acids such as 37% phosphoric acid or 10% itaconic acid with maleic acid, and they are applied over the dentin. The conditioning effect produces an increase in the size of the dentinal tubules and an increase in moisture, thereby facilitating adhesion (Ribeiro et al). This stimulus generates a nonsignificant vasodilation effect on the pulpal blood vessels neighboring the demineralized tissue (Iványi et al 2006), probably due to the release of somatosensory neuropeptides such as SP and CGRP, which bind to the NK1 and CGRP-R1 receptors in blood vessels as a defense mechanism of the dental pulp (Caviedes-Bucheli et al 2008c).

When the demineralization times are increased to 30 or 60 seconds, a significant vasoconstriction occurs in the dental pulp in the area adjacent to the demineralized dentin. The vasoconstriction is probably due to the activation of sympathetic NPY-IR fibers that compete for receptors with the somatosensory fibers in the blood vessels (Haug et al) and generate an immediate failure in the pulpal microcirculation that leads to blood flow stasis in that area. Thus, the exposure time to acid is an important factor that impacts the pulpal microcirculation of the area adjacent to the demineralization (Iványi et al). This can lead to immediate hyperalgesia, destruction of nerve fibers and blood vessels, and a neurogenic inflammatory reaction in the area (Caviedes-Bucheli et al 2008c).

The effect of conditioners and dentin bonding agents on pulpal neural response is linked to the permeability of dentin after the demineralization process, because this can be affected by the presence or absence of a smear layer. When using total-etch adhesive systems, removal of the smear layer with demineralizing acid is proposed to obtain stronger bond strengths (Bouillaguet). These compounds use 37% phosphoric acid to remove the smear layer and demineralize the underlying dentin, which may explain a greater penetration of HEMA monomers into the dentinal tubules when the dentin thickness is less than 0.5 mm (Costa et al 2000, Cetingüç et al) and consequently a greater expression of SP by dental pulp cells (Caviedes-Bucheli et al 2010). Alternatively, self-etch adhesive systems use an acidic resinous monomer that is not strong enough to dissolve the smear layer. Instead, it etches through the smear layer and demineralizes the subsurface layer of intact dentin to a depth of 0.5 μm (Tay and Pashley), generating less SP expression by the somatosensory fibers of the dental pulp when the dentin thickness is at least 0.5 mm. If a good thickness of dentin is respected over the dental pulp, self-etch adhesive systems are considered safe compared to total-etch adhesive systems (Jontell et al). No significant differences are found between these adhesive systems related to HEMA diffusion through dentin (Cetingüç et al) and pulpal tissue inflammation (Nayyar et al).

After dentin conditioning, dentin bonding agents release free radicals, such as $O_2^-$ and $H_2O_2$, through the diffusion of unpolymerized HEMA monomers. Unreacted free radicals diffuse through the dentinal tubules into the pulp due to their low molecular weight and hydrophilic characteristics (Gerzina and Hume) and can cause cell damage if pulpal defense mechanisms such as antioxidant enzymes superoxide dismutase (SOD), catalase, and glutathione S-transferase are depleted (Gallorini et al). These monomers affect the underlying odontoblastic layer, altering the cell cycle and generating inflammatory changes in the pulp (Bouillaguet et al 1996, Bouillaguet et al 2000).

Unpolymerized monomers generate ROS, which are essential signaling molecules in the activation of cellular

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aging processes, apoptosis, genotoxicity, and neurogenic inflammation through somatosensory neuropeptides such as SP and CGRP (Wang et al). The HEMA monomer can react with the antioxidant glutathione (GSH) molecule to prevent the formation of free radicals. However, when the amount of monomer exceeds the tolerance level of the pulp cells, a depletion of GSH from the cells occurs, causing apoptosis through stress-activated protein kinases (SPKs) JNK and p38 (Gallorini et al). It has been shown that SP induces the generation of ROS through activation of the enzyme NADPH oxidase, which degrades the extracellular matrix and collagen by activating matrix metalloproteinase (MMP) secretion by SP-activated cells, such as endothelial cells, fibroblasts, and inflammatory cells (Block et al), potentiating pain and neurogenic inflammation.

Self-etch dentin bonding agents increased SP release more than total-etch bonding agents when placed on cavity preparations with 0.5-mm-thick remaining dentin (Caviedes-Bucheli et al 2010). The greater release of SP in one-step self-etch bonding agents may be due to their acidic behavior and hydrophilic nature; cytotoxicity may be a consequence of an acidic change in pH. The highly hydrophilic nature of the monomers favors penetration into the hybrid layer and dentin. Self-etch bonding systems could penetrate not only intertubular dentin but also dentinal fluid-filled tubules. This liquid could interfere with the polymerization of the acidic monomer and increase its penetration, which in turn would generate greater pulpal inflammation (Nayyar et al). Acid removal can reduce cytotoxicity, and moisture can be better controlled with the use of total-etch adhesives; when the dentinal tubule is open, excess moisture can be better removed with a dentin conditioner (Bouillaguet).

Polymerization is not an instant process, as cross-linking reactions continue even after the light source has been removed. Diffusion of HEMA through dentin occurs mostly during the first few minutes after photopolymerization and is believed to be caused by its low molecular weight (greater diffusion), its conversion factor (unpolymerized monomers), and residual water from the hybrid layer and dentinal tubules that could interfere with polymerization and increase the amount of unpolymerized HEMA (Cetingüç et al). This does not mean that other more hydrophobic molecules with higher molecular weight, such as bisphenol A–diglycidyl-methacrylate (Bis-GMA), cannot penetrate to the dental pulp. After all, taxi molecules like albumin present in the dentinal fluid could transport monomers without polymerizing through the dentin to the pulp (Mahdhaoui et al). The high and early release of HEMA indicates that the monomer could remain in the dentinal tubules even after polymerization of the adhesive resin (Hamid and Hume). It has been reported that the adverse effects of adhesive restorations are evident most often during the first year after treatment, with irreversible pulpitis and pulpal necrosis being the most common adverse consequences (Whitworth et al).

SP is released immediately after release of HEMA into the dental pulp. However, it cannot be ensured that SP levels behave in the same way in the long term. It has been found that the release of methacrylate ions continues up to 30 days after placement of the restoration (Polydorou et al). Therefore, it is probable that in the long term, the expression of SP may be affected by the prolonged release of monomers (Whitworth et al, Cetingüç et al) due to the apoptotic effect of the free radicals in the pulpal tissue, triggering a chronic inflammatory response that can reduce the capacity of the pulp to defend itself from future injuries (Caviedes-Bucheli et al 2010).

In addition, light-curing units generate heat that raises the temperature of dental tissues, causing an inflammatory pulpal reaction (Savas et al). It has been shown that an increase of 42.08°F/5.6°C causes pulpal necrosis in about 15% of pulps (Kodonas et al). Factors such as power, distance from the light-curing unit to the material, exposure time, and thickness of the remaining dentin directly affect the temperature rise during the curing procedure (Santis et al). When teeth are healthy, pulp tissue homeostasis functions normally, so the integrity of the microcirculation can prevent an increase in pulp temperature because the nervous system of the pulp tissue, through its sensory, sympathetic, and parasympathetic fibers, plays an important regulatory role through the expression of neuropeptides such as SP, CGRP, NPY, and VIP (Caviedes-Bucheli et al 2008c). Teeth with pulpal inflammation due to caries lesions can be downregulated by decreasing microcirculation in an attempt to reduce heat through microcirculation (Kim 1990). Therefore, although there are many stimuli that can be manifested as heat, the adverse impact of light irradiation and the exothermic reaction of polymerization on the pulp tissues is minimal; however, this may explain why self-etch dentin bonding agents generate higher expression of SP (Fig 4-26) (Caviedes-Bucheli et al 2010).

The biologic effects of cumulative lesions in the dentin-pulp complex should not be considered separately, because the consequences derived from one factor are practically impossible to separate from the combined effects of all other factors (Mjör and Ferrari). Therefore, it is important for the clinician to have good control of each variable of the procedure carried out (Caviedes-Bucheli et al 2010).


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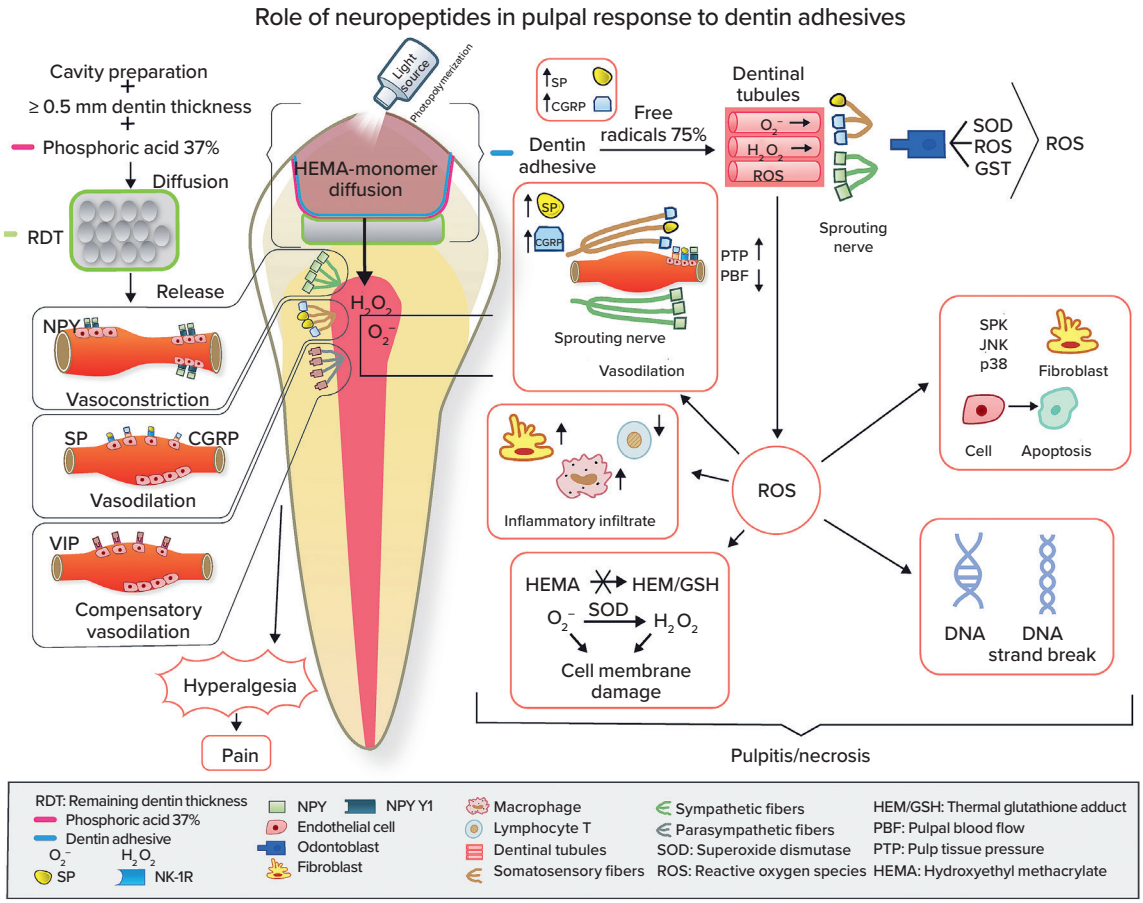


▲ **FIG 4-26** The pulpal response to dentin adhesives begins with a cumulative effect from the cavity preparation, followed by the placement of an etching agent such as phosphoric acid. This allows the diffusion of HEMA free radicals during the process of photopolymerization (of the monomer to polymer of the dentin adhesive), triggering the release of neuropeptides such as SP and CGRP from somatosensory fibers, NPY from sympathetic fibers, and VIP from parasympathetic fibers, producing vascular effects such as transient vasoconstriction induced by NPY to prolonged vasodilation produced by SP and CGRP. These reactions generate vascular congestion regulated by the expression of VIP that modulates the pulpal blood vessels with a compensatory vasodilation mechanism. This occurs when there is at least 0.5 mm of remaining dentin thickness. There is release of free radicals ($O_2^-$, $H_2O_2$) with a conversion factor not exceeding 75%. They reach the pulp through the dentinal tubules as ROS, where they overcome the defense systems of the pulp, such as the enzyme SOD, catalase, and glutathione S-transferase (GST). Disorganization of the odontoblastic layer occurs around the inflamed tissue. The increase in SP and CGRP expression induces vascular changes, raising the intrapulpal pressure (PTP) and lowering the PBF. An inflammatory infiltrate with an increase in cells such as macrophages and a decrease in T cells also occurs. The HEMA monomer reacts with the antioxidant molecule GSH to prevent the formation of ROS. When the monomer level exceeds the tolerance of the pulp cells, depletion is generated, damaging the cell membrane and causing cell apoptosis through the SPK pathway such as p38 or JNK, thereby generating DNA damage. Clinically, this reaction can lead to stages of pulpitis or necrosis.

### Luting cements

Restorative dentistry also requires the use of luting cements (Ergin and Gemalmaz). Glass-ionomer cements are used as luting agents in dentistry; however, they have a relatively slow setting, which makes them very sensitive to moisture that reduces their strength and durability (Smith and Ruse). Clinically, they are associated with postoperative sensitivity related to their low pH (less than 3) during the first 24 hours (Ergin and Gemalmaz). Resin-based cements are widely used today, claiming to have better mechanical properties than other cements. They behave in the same way as dentin bonding agents in polymerization processes, whereby free radicals are released, and are therefore associated with some types of hyperalgesia, ranging from dentinal hypersensitivity to symptomatic irreversible pulpitis (Caviedes-Bucheli et al 2008c).

Dentin thickness plays a crucial role in the response of the pulp tissue to the use of cementation materials. Resin-based cements have been shown to generate significantly higher SP release compared to glass ionomers. This could be explained by the ability of HEMA monomers to diffuse through dentin, as they have a smaller particle size than

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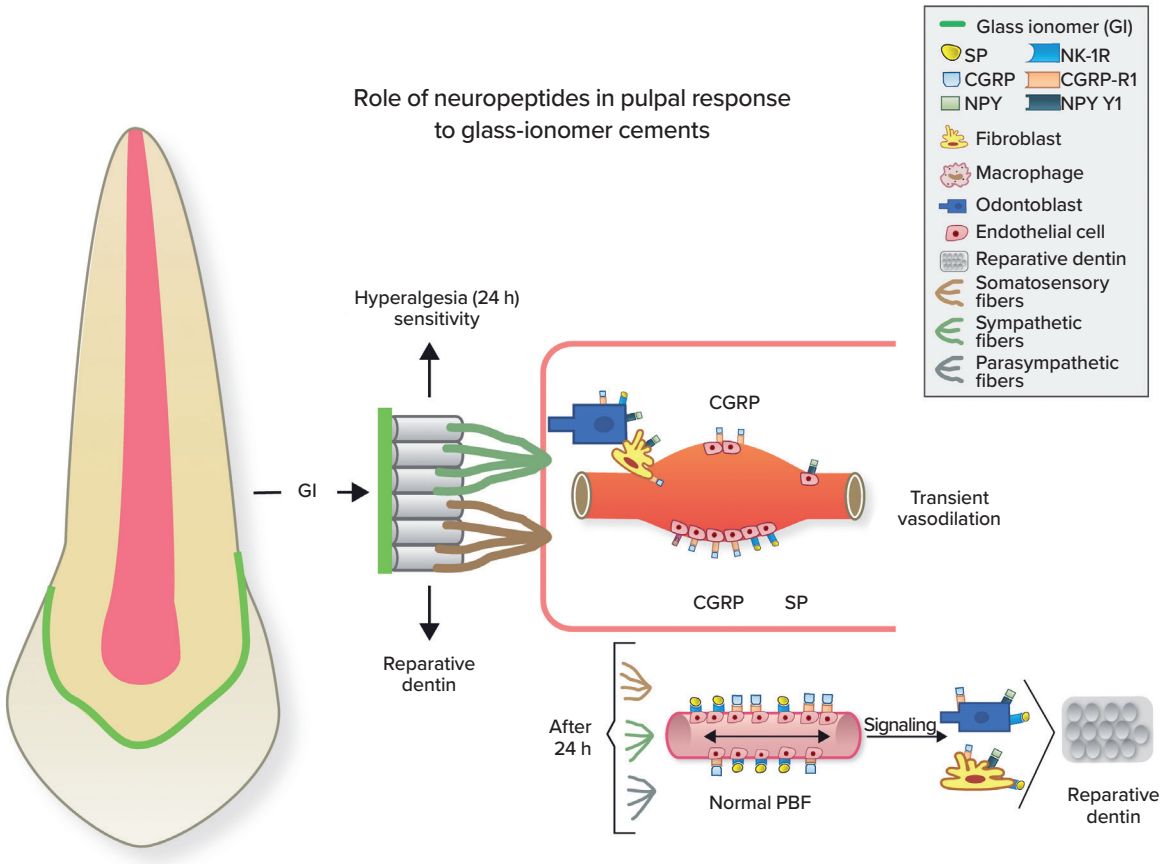


▲ **FIG 4-27** The pulpal response to cementation agents, such as glass-ionomer (GI) cements, generates hyperalgesia that clinically translates into dentinal hypersensitivity in the first 24 hours. It occurs as a consequence of neurogenic inflammation where somatosensory neuropeptides generate transient vasodilation regulated by the neuropeptides from sympathetic fibers (eg, NPY) and parasympathetic fibers (eg, VIP). These reactions contribute to maintaining normal PBF, allowing signaling of odontoblasts and fibroblasts through specific receptors, such as NK-1R, CGRP-R1, NPY Y1, and VPAC1, to induce the formation of a hard tissue such as reparative dentin, preserving pulp vitality.

glass-ionomer cements, which allows greater diffusion of the monomer through dentinal tubules and generates the same reaction observed with dentin bonding agents (Gerzina and Hume).

SP expression after cementation of restorations with glass-ionomer cements may be due to the slow setting of glass ionomer, which involves an acid-base reaction, making it very sensitive to moisture. This slow setting constitutes a considerable aggression to the pulp during the first 24 hours, while the chemical reaction and the pH of the cement stabilize (Aranha et al 2006). After the first day, glass-ionomer cement is very well tolerated by the dentin-pulp complex, constituting a very good option as a cementation material for indirect restorations in terms of respecting pulpal health (Fig 4-27) (Caviedes-Bucheli et al 2013).

## Neural reactions to tooth-bleaching agents

The demand for esthetic procedures has increased considerably in contemporary dentistry, and teeth whitening is one of the most requested clinical procedures by patients (Gallinari et al). There is evidence in the literature that the chemicals used for this procedure have deleterious effects on the dentin-pulp complex (Marshall et al). Chemical products used for teeth whitening usually contain some form of hydrogen peroxide ($H_2O_2$) in different concentrations or related substances such as sodium perborate or carbamide peroxide. Carbamide peroxide, for example, can generate 7% urea and 3% $H_2O_2$ in a hydrophilic environment (Goldberg et al).


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Once in contact with organic or inorganic compounds, $H_2O_2$ generates ROS such as hydroxyl ($OH^-$-), perhydroxyl ($HO_2^-$), and oxygen ($O_2^-$), among other free radical ions containing unpaired electrons, and through redox reactions it generates a rupture of chromogenic molecules (chromophores) and a change in energy absorption within the pigmented dental organic matrix, forming simpler and smaller molecules that reflect light differently, thereby creating a whitening effect. This effect can also be accelerated by chemical or physical activators (ie, light, enzymes, or heat) (Li, Goldstein). ROS have a low molecular weight, so they are able to penetrate the enamel and diffuse through the organic matrix of dentin to exert their whitening effect (Gökay et al). The presence of dentinal tubules within the dentin architecture favors these free radicals to reach the pulp tissue and generate damage at the cellular, vascular, and nervous levels (Bowles and Ugwuneri, Cooper et al 1992).

These highly reactive free radicals have been associated with various physiologic and pathologic events, including the degradation of proteins, lipids, and nucleic acids, leading to tissue aging and other degenerative processes (Li, Sun). Due to their high oxidative power, they can break the double bonds of inorganic and organic compounds in the enamel, dentin, and dental pulp, generating chemical alterations in the mineralized tissues and tissue damage in the pulp (Redha et al, Toledano et al).

The release of free radicals to achieve a tooth whitening effect is dependent on concentration, time, and activation method. The higher the concentration of $H_2O_2$ and the longer the application time, the greater release of free radicals and therefore greater whitening efficiency but also greater oxidative stress on dental tissues. Moreover, if tooth-bleaching agents are activated with light, heat, or enzymes, even more free radicals are released, and the damage to the dentin-pulp complex will be proportional to their release (Caviedes-Bucheli et al 2008a, Min et al).

The enamel constitutes the first line of defense to protect the pulp against free radicals. It is a highly mineralized tissue, with 95% of its volume occupied by hydroxyapatite (HA) crystals organized into prism-like structures called *enamel rods*. The space between each rod contains small crystals of HA and organic material through which free radicals diffuse (due to their low molecular weight and their ability to denature proteins) and thereby reach the dentin, which constitutes the second mineralized defense layer (Robinson et al, Haywood). When ROS radicals penetrate the dentinal tubules, the dentin structure becomes more permeable, but they still have to overcome different defense mechanisms of the pulp, such as the odontoblastic process, dentinal fluids, and collagen fibers, which act as a biologic barrier by interacting with these molecules (due to the high reactivity of free radicals) (Puapichartdumrong et al). In addition, the positive intrapulpal pressure and the osmotic pressure of the gel could oppose the diffusion of radicals toward the pulp (Benetti et al).

In the event that free radicals reach the pulp tissue, the pulp cells have the ability to synthesize enzymes such as catalase, glutathione peroxidase (GPx), and SOD, which are part of the body's homeostatic system and serve as a defense mechanism against peroxides and free radicals. SOD catalyzes the dismutation of the superoxide radical $O_2^-$, converting it into $H_2O_2$. Although $H_2O_2$ is not a free radical, it can be rapidly converted by a Fenton reaction to the highly reactive hydroxyl radical $OH^-$. The enzyme GPx neutralizes this hydrogen peroxide by taking hydrogen from two molecules of GSH, generating water and glutathione disulfide (GSSG). Finally, the GPx regenerates GSH from GSSG. It has been shown that this normal balance between free radicals generated during normal cellular metabolism and the enzymes mentioned above is affected by the presence of exogenous $H_2O_2$, which penetrates the cell membrane and does not allow these enzymes to carry out their work inside the cell. For example, a significant increase in free radicals and a depletion of intracellular GSH stores have been observed in human oral fibroblasts exposed to $H_2O_2$ (Engelmann et al).

Once GSH stores have been depleted due to continuously increasing $H_2O_2$ concentrations, cell proliferation is inhibited by DNA strand breakage and oxidative damage to DNA bases (eg, 8-oxoguanine), which generates biologic damage (Hanks et al). These events can alter the defense capacity of the pulp, generating apoptosis phenomena, which has already been demonstrated by observing increased presence of apoptotic cells in cultures exposed to high concentrations of $H_2O_2$ (Burdon et al).

When highly reactive radicals ($OH^-$ and $O_2^-$) penetrate the dentin-pulp complex, odontoblasts and pulp fibroblasts generate the synthesis of heme oxygenase-1 (HO-1), which increases after cell exposure to oxidative stress (Anderson et al). HO-1 is a heat shock protein that responds rapidly to insults that threaten cellular homeostasis. Despite this, in an environment of low adaptability such as the dental pulp, this type of aggression could generate an inflammatory response and induce pulp cells (including odontoblasts, fibroblasts, and undifferentiated mesenchymal cells) to undergo apoptosis, which includes programmed cell aging, reducing the capacity of the tissue to defend itself against future irritants (Bowles and Thompson).

The odontoblasts and their processes are the first cells that come into contact with the free radicals of tooth whitening (Soares et al). These cells are of vital importance because they are involved in the initiation, development, and maintenance of the inflammatory/immune response of the pulp (Farges et al). Odontoblasts have

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shown strong positive staining for HO-1, which could in part be responsible for the apparent safety of the tooth whitening mechanism that is altered by the action of free radicals (Anderson et al).

Significant concentrations of $H_2O_2$ have been demonstrated in the pulp chamber after the application of whitening gel to dental enamel (Soares et al), generating morphologic changes and a decrease in the mitochondrial respiration rate in odontoblastic cells, which leads to a loss of viability and to apoptosis processes (Gallinari et al). In vivo studies have even shown areas of coagulation necrosis in human mandibular incisors and disruptions in the odontoblastic layer of pulp tissue of human premolars (Kina et al).

Fibroblasts constitute the second line of pulpal defense; it has been shown that these cells are capable of generating SOD as a defense mechanism during inflammatory processes (Skaleric et al) and that GPx and catalase help to protect fibroblasts from high concentrations of $H_2O_2$ (Masaki et al). These three enzymes together with glutathione reductase (GR) constitute the main enzymatic defense pathways of all aerobic cells against free radicals. Despite this, $H_2O_2$ is toxic to these cells when released in large amounts, inhibiting cell proliferation by breaking the DNA chain and generating biologic damage, which alters the defense capacity of the pulp due to apoptosis (Hanks et al). Finally, the third line of defense is provided by the mesenchymal stem cells of the pulp, which are recruited and then proliferate and differentiate into cells similar to odontoblasts, allowing the regeneration of the dentin-pulp complex (Kerkis et al, Lizier et al).

Cellular damage caused by the penetration of $H_2O_2$ induces the synthesis and release of biochemical mediators, such as prostaglandins, histamine, and bradykinin, initiating an inflammatory process (Cintra et al). These mediators cause an increase in vascular permeability and vasodilation within the pulp cavity (Soares et al), stimulating peripheral nerve fibers, which respond with the production and release of SP and CGRP. Sympathetic NPY and parasympathetic VIP are also released (Caviedes-Bucheli et al 2008c). These neuropeptides activate transmission neurons, promoting pain signaling and inflammation from the injured tissue (Harrison and Geppetti, Almeida et al). Therefore, during tooth whitening procedures, patients usually suffer some type of clinical symptomatology, from dentinal hypersensitivity to reversible or irreversible inflammatory processes (Caviedes-Bucheli et al 2008a).

Moreover, when using 25% and 35% $H_2O_2$ with light/heat activation (ie, ultraviolet light and infrared laser diode), SP levels are significantly increased compared to baseline levels and bleaching with 38% $H_2O_2$ without activation. The high levels of SP found with the use of $H_2O_2$ activators may be clinically relevant because they could trigger a neurogenic inflammatory reaction in the pulp tissue due to the degenerative processes that free radicals produce over time in cells, nerve fibers, and blood vessels (Caviedes-Bucheli et al 2008a), resulting in damage to the pulp tissue and release of cell-derived factors such as adenosine triphosphate (ATP) and prostaglandins, which excite or sensitize pulp nociceptors (Goodis et al, Cook and McCleskey). Activation of these nociceptors leads to the release of vasoactive peptides in the vicinity of the pulpal blood vessels and sympathetic peptides with significant neural sprouting at the pulp and dentin levels (Caviedes-Bucheli et al 2008c).

Therefore, the clinical manifestation of postbleaching sensitivity (pain) is one of the defense mechanisms of the pulp, which may be confused with dentin sensitivity. The difference between the two is difficult to explain because the dentin is sealed by the enamel without any dentin exposure, differing from the concept of dentinal hypersensitivity because the mechanism of pain generation is different (Markowitz). The expression of SP and CGRP as a consequence of the presence of free radicals by bleaching procedures generates an inflammatory reaction in the dental pulp, mostly of a reversible nature, which may generate dentin sensitivity due to the movement of liquids in the dentinal tubules that excite the Aβ-, Aδ-, and C-fibers in an antidromic direction (ie, from the inside out). This explains the postbleaching sensitivity, which cedes spontaneously when the pulp inflammation reduces (Fig 4-28) (Caviedes-Bucheli et al 2008c, He et al).

## Neural reactions to orthodontic movement

Orthodontics is the only specialty of dentistry that uses the inflammatory process as a therapeutic modality to improve and solve the functional and esthetic problems of the masticatory system. There are different types of orthodontic forces that can be applied to achieve dental movement, including intrusion, extrusion, rotation, inclination, and torquing movements. Depending on the type of movement required, a certain amount of grams times force is applied to the tooth to achieve its displacement (Javed et al).

The pulpal response to the application of an orthodontic force implies that inflammation of a neurogenic nature is generated, reflected by the expression of neuropeptides such as SP, CGRP, and NKA (Caviedes-Bucheli et al 2008c). Following this inflammation, there are cellular and enzymatic alterations, aspiration and vacuolization of pulp cells, disruption of the odontoblastic layer, a decrease of alkaline phosphatase (ALP) activity, an increase of aspartate aminotransferase (AST) activity, and activation of apoptosis mechanisms (Hamilton and Gutmann). Also, vascular


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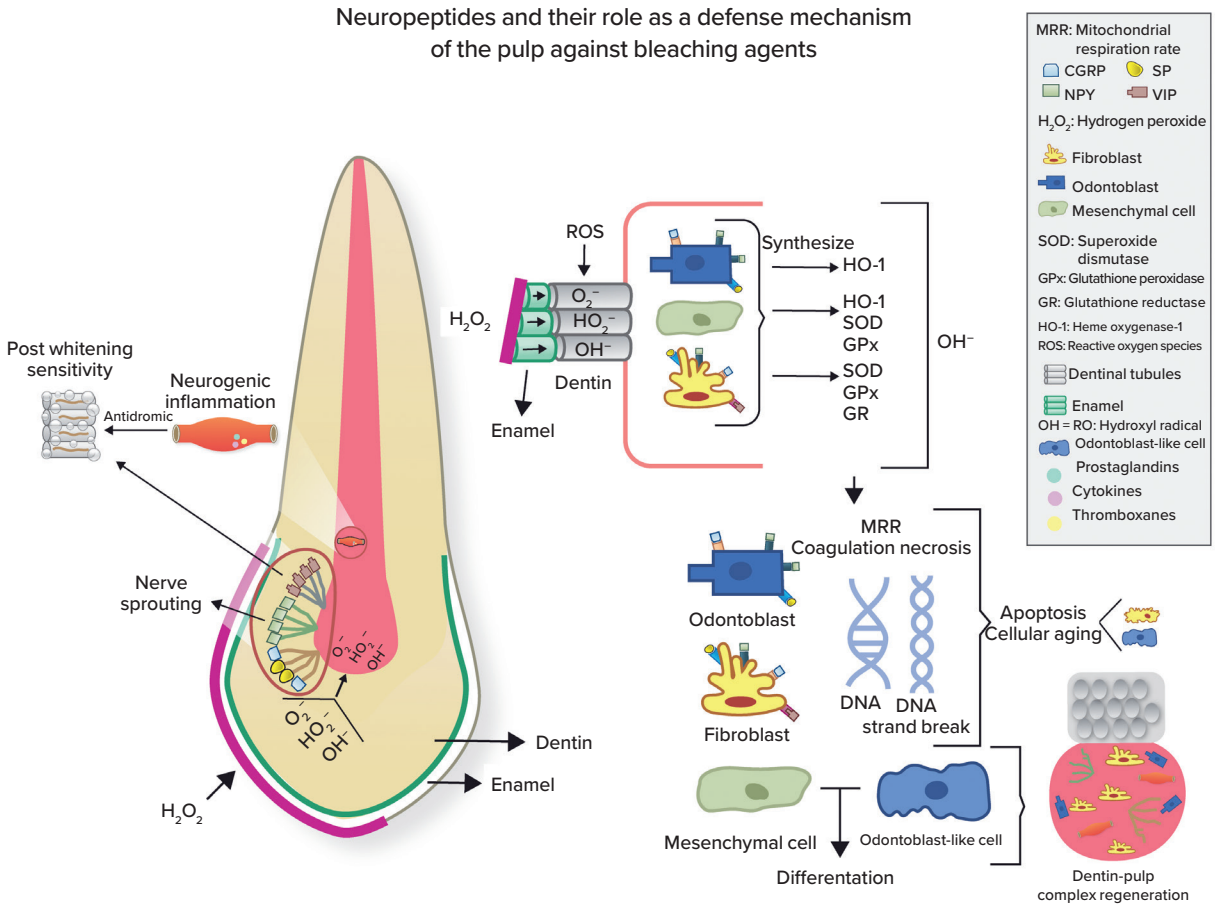


▲ **FIG 4-28** $H_2O_2$ is the most commonly used agent for tooth whitening. It generates free radicals such as $OH^-$, $HO_2^-$, and $O_2^-$, considered ROS, which have a low molecular weight and can penetrate the enamel and dentin to reach the pulp tissue. The pulp cells have the capacity to synthesize enzymes such as GPx, SOD, and HO-1. The fibroblasts release HO-1, SOD, and GPx, while the odontoblasts release HO-1 as a defense mechanism against free radicals. When free radicals are released in large quantities and exceed the tolerance of the pulp, they inhibit cell growth and reduce the rate of mitochondrial respiration, producing coagulation necrosis that leads to DNA breakage, thereby triggering apoptosis and cell aging mechanisms. This cell damage can generate a neurogenic inflammatory process with the release of neuropeptides such as SP, CGRP, NPY, and VIP from the somatosensory, sympathetic, and parasympathetic nerve fibers, generating reversible episodes of pain and inflammation clinically represented as dentin sensitivity. This is manifested due to dentinal fluid movements in the dentinal tubules in an antidromic direction (from the inside out) that excite the Aβ-, Aδ-, and C-fibers. This explains the postbleaching sensitivity that spontaneously subsides when the pulp recovers.

changes take place, including altered pulpal microcirculation, decreased tissue respiration rate, hypoxia, tissue damage with an increased number of fibers and decreased cellular component that induce tissue fibrosis (Grünheid et al), and a defense response by angiogenic mechanisms that lead to pulpal calcifications or pulpal aging (Caviedes-Bucheli et al 2017, Caviedes-Bucheli et al 2021). It has been shown that depending on the duration, type, and magnitude of the force, as well as physiologic tissue tolerance and dental age, these alterations can affect the pulp tissue reversibly or irreversibly (Caviedes-Bucheli et al 2011b).

The first change that occurs in pulp tissue when there is an orthodontic movement is the disorganization of the odontoblastic layer. This alteration ranges from simple disorganization in the initial stages of movement (after application of mild or moderate forces) to vacuolization of pulp cells that lead to enzymatic alteration of the cells, generating irreversible apoptotic reactions or cell aging, especially after severe force application with a long-lasting duration that exceeds the tolerance of the pulp tissue (Veberiene et al). Within the enzymatic alterations at the cellular level in the pulp, a reduction in the activity of ALP (Perinetti et al 2005) and an increase in the activity of AST have been found, which are related to the magnitude and time of force application (Perinetti et al 2004). Fibroblasts and odontoblasts synthesize and release ALP (Spoto et al), which is considered a marker of the proper functioning of the defense mechanism of these cells, as ALP activity is reduced due to stress generated by mild or moderate orthodontic forces (Fig 4-29) (Perinetti et al 2005).

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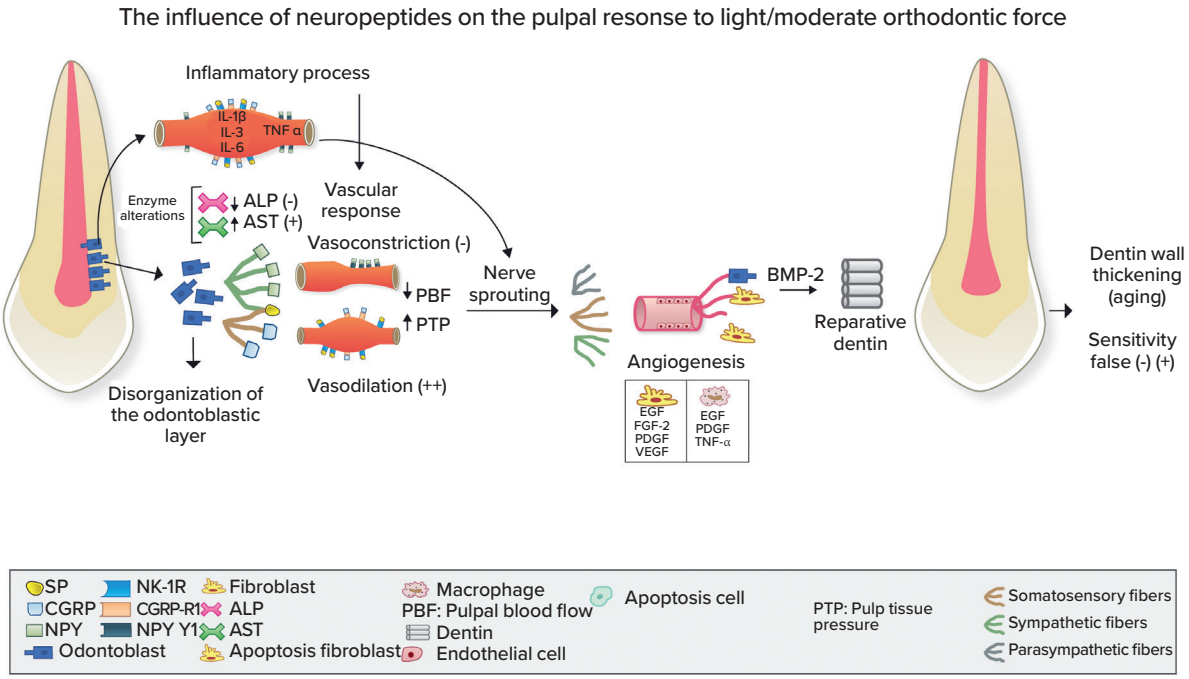


▲ **FIG 4-29** The pulpal response to mild/moderate orthodontic force begins with the disorganization of the odontoblastic layer, with cell vacuolization processes that generate an enzymatic alteration in cells such as odontoblasts and fibroblasts. There is a reduction in ALP and an increase in the activity of AST, which is related to cell damage due to the magnitude and time of application of force. When mild and moderate forces are applied, the damage is reversible and susceptible to repair. A neurogenic inflammatory reaction is generated where nerve fibers express neuropeptides such as SP, CGRP, NPY, and VIP that induce the accumulation of inflammatory mediators such as IL-1β, IL-3, IL-6, and TNF-α from inflammatory cells, in addition to a vascular response that generates a decrease in PBF and an increase in pulp tissue pressure (PTP). As a defense mechanism, there is sprouting of nerve fibers that could release neuropeptides and stimulate the formation of reparative dentin. This is evidenced radiographically as a decrease in the lumen of the dental pulp and clinically with inconclusive sensitivity tests.

The release of angiogenic growth factors such as EGF, VEGF, FGF-2, PDGF, transforming growth factor-β (TGF-β), and TNF-α demonstrates that pulp cells undergo damage during the early phases of orthodontic treatment (Derringer and Linden 2003, Tran-Hung et al 2008). Moreover, the increase in the levels of the enzyme AST, which is a biologic marker of cellular necrosis, indicates tissue damage. If force is not controlled, it could lead to cell death, which generally occurs when severe orthodontic forces are applied (Von Böhl et al).

Depending on the magnitude of the force, more changes can occur at the vascular level, ranging from an alteration of the pulpal microcirculation in the initial stages of the orthodontic movement to significant damage to the supply of PBF when severe forces are applied. This has a direct impact on the metabolism of the pulp tissue, generating initial alteration in the blood vessels of the apex that later ends up involving the entire pulp (Ramazanzadeh et al, Weissheimer et al).

The effects of orthodontic forces on PBF are associated with several factors, including the age of the patient, the size of the apical foramen, dentinogenic activity, and the magnitude and duration of the applied force (Weissheimer et al, Javed et al). In teeth with a closed and mature apex, the alteration of blood flow is greater than in teeth with open and immature apices when forces of a moderate to severe range are applied. Time elapsed will also greatly influence the behavior of the pulp microcirculation (Han et al). For example, when an intrusive force is applied to incisors and premolars, PBF is affected for 72 hours to 3 weeks, while in molars the effect lasts for 3 months. This happens because the molars have smaller apical foramina that further restrict the blood flow that enters through the foramina toward the dental pulp (Vitali et al). Depending


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on the magnitude and time of activation of the force, this alteration in the PBF could generate an increase in the deposition of reparative dentin at the coronal or radicular level, generating a partial and/or total obliteration of the pulp chamber and the root canal in teeth with complete root formation subjected to moderate forces (Hamilton and Gutmann). When light forces are applied for short periods of time, blood flow undergoes little alteration, with a slight reduction in PBF followed by a return to average values (Barwick and Ramsay, Abu Alhaija et al 2019, Abu Alhaija and Taha 2021).

During orthodontic movements, the pulpal microcirculation undergoes dynamic changes that compromise its ability to remove metabolic waste products and maintain a harmonized interstitial pressure. Consequently, in some cases edema and necrosis of the pulpal tissue can occur (Kim 1990). Studies using laser Doppler flowmetry (LDF) confirmed a temporary decrease in PBF associated with a drop in oxygen pressure, generating areas of hypoxia and increasing the possibility of cell injury and apoptosis (McDonald and Pitt Ford). When moderate orthodontic forces are applied, this damage can usually be reversed through angiogenic mechanisms and dentin formation as a defense mechanism (Caviedes-Bucheli et al 2021). When severe forces are applied, the reduction of PBF is more pronounced, with significantly more irreversible alterations in the pulpal tissues (Han et al). It is for this reason that pulp sensitivity should be monitored on a regular basis during orthodontic treatment.

The application of orthodontic force alters the pulpal response to sensitivity tests. If the forces are applied correctly and the tolerance of the pulp is not exceeded, an initial increase in sensitivity with a progressive normalization occurs. This initial increase is explained by a hypoxic microenvironment that alters the response threshold of the Aδ-fibers located more peripherally in the dental pulp. An increase in negative responses to sensitivity tests has also been observed in teeth with active orthodontic force applied; however, most of the time the tooth begins to respond positively after a period of rest, and necrosis usually does not occur (Vitali et al).

The main defense mechanism of the dental pulp in response to orthodontic movements is the formation of mineralized tissue. In order to make this possible, angiogenic mechanisms take place in dental pulp (Caviedes-Bucheli et al 2020). The stress produced in the pulp tissue by orthodontic forces generates vascular and cellular alterations characterized by a high demand for oxygen by the pulp cells to compensate for the hypoxia generated due to the altered PBF following orthodontic movements (Sakdee et al, Aranha et al 2010). Therefore, new blood vessels must be formed at the expense of existing ones, which suggests an increase in angiogenic growth factors (Caviedes-Bucheli et al 2021, Gomez-Sosa et al 2021).

Gene expression of several angiogenic growth factors has been identified in the dental pulp of teeth with complete and incomplete root formation, such as VEGF, FGF-2, PDGF, angiogenesis 1 (ANG-1) and 2 (ANG-2), and TGF-β (Gomez-Sosa et al 2019); this activates the classic and alternative angiogenic pathways in response to this type of stimuli (Caviedes-Bucheli et al 2020), which leads to the formation of new blood vessels at the expense of existing ones. In the classical pathway, VEGF guides the process (Caviedes-Bucheli et al 2021), and through an alternate pathway ANG-1 and ANG-2 exercise that function (Caviedes-Bucheli et al 2020). This is the kickstart for the formation of new blood vessels to supply the lack of oxygen that fibroblasts, odontoblasts, and stem cells are undergoing in order to start forming mineralized tissue as a defense mechanism (Caviedes-Bucheli et al 2017, Gomez-Sosa et al 2021). Orthodontic movement also modifies the expression of proteins that form the extracellular matrix by stimulating fibronectin, collagen, and VEGF. These first two molecules are synthesized by pulp fibroblasts as a reparative response to the application of orthodontic force, and their levels return to normal 30 days after the force is applied (Vermiglio et al).

These cellular and vascular changes occur as a consequence of a neurogenic inflammatory reaction of the dental pulp in response to orthodontic movements, where the progression of the inflammatory process apparently depends on the production of inflammatory cytokines such as IL-1α, IL-1β, IL-3, IL-6, and TNF-α (Von Böhl et al) and stimulation by neuropeptides such as SP, CGRP, and NKA (Caviedes-Bucheli et al 2008c, Caviedes-Bucheli et al 2021). If the orthodontic force is controlled, the damage to the pulp tissue is reversible, but if the force or the movement is not controlled, the stimulation of these neuropeptides and cytokines will end up causing an alteration in the PBF, generating irreversible damage such as pulpal calcifying degeneration, internal root resorption, or necrosis (Fig 4-30) (Yamaguchi et al, Von Böhl et al).

Neuropeptides are released according to the magnitude of the force and the type and duration of movement, generating changes at the cellular and vascular level (Ren et al, Caviedes-Bucheli et al 2011b). CGRP and SP are capable of triggering vasodilation, plasma extravasation, activation of the immune system, chemotaxis, and recruitment and/or regulation of inflammatory cells such as macrophages, mast cells, and lymphocytes (Caviedes-Bucheli et al 2008c). In teeth subjected to moderate orthodontic forces, there is no significant increase in CGRP and SP levels, which can be correlated with less damage to the pulp tissue (Caviedes-Bucheli et al 2011b). Consequently, there is less alteration in PBF, which allows the

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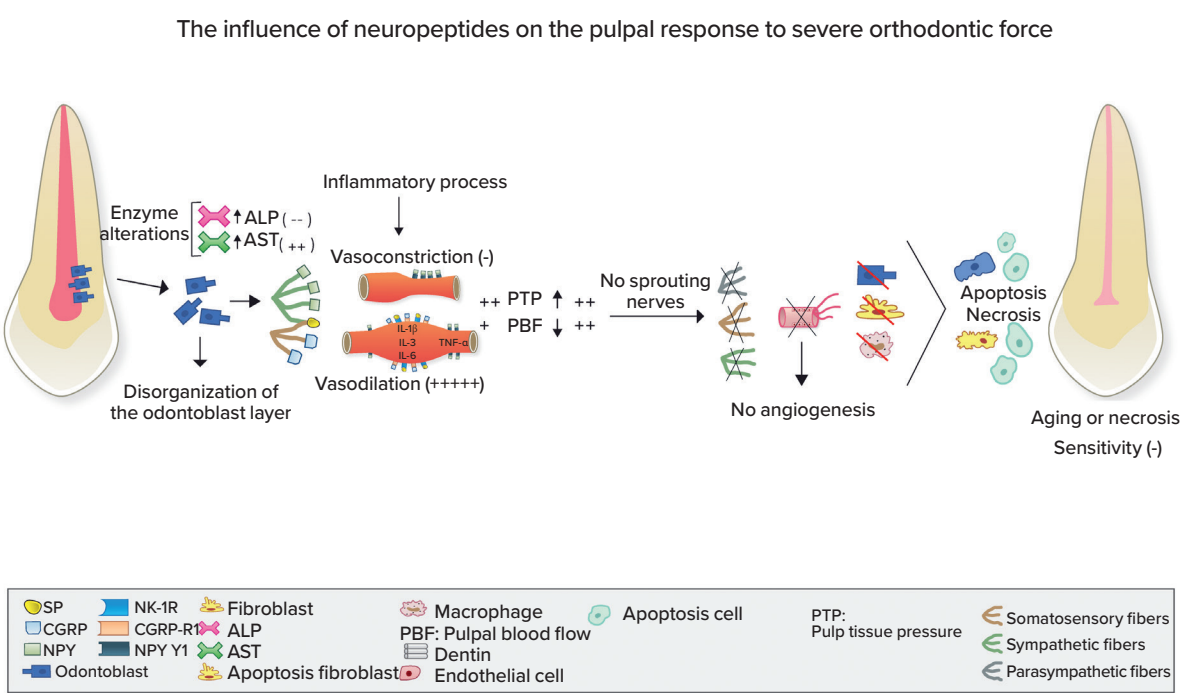


▲ **FIG 4-30** When severe orthodontic force is applied, the enzymatic damage is greater, with higher expression of AST and ALP, an inflammatory reaction that leads to vascular stasis without nerve sprouting or angiogenesis, finally leading to apoptosis and necrosis of the pulp cells, which clinically is evidenced as pulpal aging or necrosis.

compensatory mechanism of releasing angiogenic growth factors into the extracellular space to take place (Caviedes-Bucheli et al 2017). These changes aim to avoid irreversible damage caused by hypoxia of the pulp tissue (Derringer and Linden 2003, El Karim et al 2009). Teeth that have been subjected to severe orthodontic forces experience a significant increase in SP and CGRP expression compared to normal neuropeptide values. This causes SP-IR and CGRP-IR sensory nerve fibers to respond to excessive orthodontic forces (Vandevska-Radunovic, Kvinnsland and Kvinnsland). In the long term, a severe orthodontic force magnifies the inflammatory process of the pulp and could lead to asymptomatic irreversible pulpitis and necrosis (Javed et al).

CGRP released by nerve fibers potentiates the action of SP, causing a significant alteration in tissue homeostasis and pain sensitivity by triggering the release of inflammatory mediators. If the orthodontic force is not removed, the nerve fibers could become sensitized and therefore experience spontaneous depolarization, which would increase the response to pain (Vandevska-Radunovic, Caviedes-Bucheli et al 2011b). SP and CGRP increase in the dental pulp suggests that orthodontic forces are capable of triggering a cellular response similar to that observed during caries progression, cavity preparation, and occlusal trauma (Caviedes-Bucheli et al 2005c, Caviedes-Bucheli et al 2011a, Caviedes-Bucheli et al 2021). In addition to the above, it has been found that CGRP amplifies the inflammatory effects of SP by increasing the release of inflammatory mediators, leading to irreversible damage and pulp necrosis if not controlled (Yamaguchi et al, Caviedes-Bucheli et al 2008c).

CGRP and SP also increase the expression of BMP-2 transcripts in human pulp cells, stimulating odontoblasts to increase dentin deposition as a defense response. This mechanism is mediated by the expression of NK1 (Caviedes-Bucheli et al 2007c) and CGRP1 receptors (Caviedes-Bucheli et al 2005a) by odontoblasts and pulp fibroblasts, which induce the pulp tissue to undergo a degenerative calcification reaction that could partially or completely obliterate the pulp space (Trantor et al, Calland et al). Therefore, it is important to point out the clinical


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relevance of using moderate and intermittent orthodontic forces, which are capable of generating adequate tooth movement while limiting damage and allowing the pulp to recover from injury (Vandevska-Radunovic, Caviedes-Bucheli et al 2011b).

## Neural reactions to occlusal trauma

The dental pulp has several functions, including hard tissue formation (ie, tertiary dentin) to compensate for tissue loss caused by mechanical trauma from masticatory function and occlusal trauma (Arana-Chavez and Massa, Cooper et al 2010). Occlusal trauma occurs when the masticatory function exceeds the tolerance of the dental pulp, generating an inflammatory process of neurogenic nature. This is caused by a dysfunction of the masticatory system or parafunctional habits like bruxism. Moreover, occlusal interferences generated by orthodontic movements are also associated with occlusal trauma (Caviedes-Bucheli et al 2011a).

Occlusal trauma generates sprouting of somatosensory fibers that release neuropeptides such as SP, CGRP, and NKA (Lundy and Linden), sympathetic fibers that release NPY, and parasympathetic fibers that release VIP (Kvinnsland et al 1989). The increase in extracellular neuropeptides induced by occlusal trauma is related to greater activity in the synthesis of the neuropeptide in the cervical and trigeminal ganglia, increase in the transport speed of the neuropeptides in the neurons, and increase in the release or decrease of peptidase levels, which would result in decreased neuropeptide degradation (Buck et al). It has been shown that mRNA transcripts are transported to peripheral terminals, suggesting that peptide synthesis could occur directly at peripheral terminals (Price et al).

When the pulp is subjected to constant and prolonged stimuli such as occlusal trauma, it undergoes an aging process where blood perfusion decreases in the presence of harmful stimuli, affecting the nutrition and oxygenation of the tissues and thereby passing from a state of normoxia, with optimal levels of oxygen, to one of hypoxia, with low oxygen levels and high levels of hypoxia-inducible factor-1α (HIF-1α). In this state, the functional cellular demand of the tissue is not adequately met by the existing oxygen levels (Caviedes-Bucheli et al 2017). Therefore, the tissue must be replenished with blood flow for the repair processes to be carried out optimally. The vascular neoformation will fulfill the function of providing oxygen and nutrients to the injured tissue to make the dental pulp viable (Ikeda et al, Tran-Hung et al 2008, Marchionni et al, Zhang et al 2011). This process is known as *angiogenesis* and is regulated by numerous growth factors such as VEGF, EGF, PDGF, FGF, and insulin-like growth factor-1 (IGF-1), as well as by SP and CGRP (Caviedes-Bucheli et al 2007a). Angiogenesis is necessary for the formation of tertiary dentin as a defense mechanism (Ikeda et al, Killough et al 2009, Saghiri et al).

The SP/NK1 and CGRP/CGRP-R1 complexes stimulate angiogenesis using direct mechanisms that modulate the action of endothelial cells by inducing mitotic activity to form new blood vessels. They also act on fibroblasts by inducing the release of growth factors such as VEGF and stimulating cell migration and proliferation to form mineralized tissue as a defense mechanism (Tran-Hung et al 2006, Killough et al 2009, Aranha et al 2010). These complexes also use indirect mechanisms, attracting cells with angiogenic potential, via binding to granulocytes or macrophages (El Karim et al 2009, Tuo et al, Caviedes-Bucheli et al 2017).

If angiogenesis is analyzed as a physiologic event, it can be inferred that it comprises two processes: *(1)* the sprouting of endothelial cells and *(2)* the division of vessels due to abnormal growth of the microvasculature. Organs derived from the ectoderm and mesoderm are vascularized by angiogenesis, a process that occurs during embryogenesis and remains as a normal condition during inflammation and tissue repair (Derringer et al 1996, Tran-Hung et al 2006, Marchionni et al). Several studies indicate that tissue repair involves the formation of new vessels, which is necessary for tissue repair to take place, where both SP and CGRP promote the differentiation and proliferation of endothelial cells (Artese et al, El Karim et al 2009, Kohara et al).

There is evidence indicating that in the presence of occlusal trauma, oxygen levels in the pulp decrease so that cell damage, vascular disorders, and inflammation can occur. Various cells induce defense and repair processes within the dentin-pulp complex, starting with odontoblasts first, followed by dental pulp stem cells (DPSCs) and human dental pulp fibroblasts (HDPFs). The latter are found in greater proportion and are responsible for mineralizing collagen matrix just before the formation of reparative tertiary dentin (Arana-Chavez and Massa, Killough et al 2009, Rosa et al). HIF-1α and VEGF expression are increased in DPSCs and HDPFs, and therefore angiogenesis takes place. This explains why the vascular neoformation plays a fundamental role in the repair of the pulp tissue, because these new vessels are able to satisfy the metabolic and oxygenation needs of the pulp, which is possible due to the mediation of the nervous system through neuropeptides such as SP and CGRP (Derringer et al 1996, Derringer and Linden 1998, Aranha et al 2010, Zhang et al 2011).

Angiogenesis is initiated as the VEGF expression level increases, creating vascular destabilization through increased HIF-1α (Mishima et al, Wei et al). VEGF binding to its receptor VEGF-R2 is responsible for the

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differentiation, proliferation, migration, and tubulogenesis of endothelial cells. Subsequently, VEGF bound to VEGF-R1 acts on stem cells and participates in the guidance and control of cell formation in the new vasculature until its stabilization to reach normoxia (Potente et al, Rombouts et al).

A unique feature of the pulp microvasculature is the presence of arteriovenous anastomoses (AVAs), which participate in regional control of blood flow due to their dilation or contraction in the presence of mechanical irritation such as occlusal trauma (Matthews and Andrew, Heyeraas and Berggreen, Rodd and Boissonade 2002). The alteration of the vasculature is reflected in vascular stasis, decreasing the resistance of the vessels and causing edema in the pulp tissue. All these changes are caused not only by local control mechanisms of the blood vessels, such as the AVAs in the apical third and the presence of pre- and postcapillary sphincters in the coronal third, but also by the main control exerted by the nervous system in the regulation of PBF. This regulation is the result of neuropeptide release from sympathetic fibers, such as NPY, that generate vasoconstriction; somatosensory fibers, such as SP, CGRP, and NKA, that generate vasodilation; and parasympathetic fibers, such as VIP, that regulate the needs of the pulp under occlusal trauma with vasodilation (Kim 1990, Olgart 1996, Rodd and Boissonade 2002, Lee et al 2013).

The basal levels of SP and CGRP in healthy pulp allow the homeostasis of the tissue, with an adequate supply of oxygen and nutrients, contributing to the correct functioning and maintenance of the pulp. It was determined that teeth that have been subjected to experimental occlusal trauma showed an increase of 45% in the levels of SP in the pulp and 49% in the levels of CGRP, supporting the hypothesis that sensory nerve fibers respond to excessive masticatory forces (Caviedes-Bucheli et al 2011a, Caviedes-Bucheli et al 2021). Moreover, mechanical irritation of the dentin could affect the dental pulp as a result of increased fluid movement within the dentinal tubules, stimulating nociceptors in the pulp (Rees). If the occlusal interference is not removed, the nerve fibers could become sensitized and thus undergo spontaneous depolarization, increasing the response to pain (Kvinnsland et al 1992, Ikeda et al).

In addition to pain control, SP and CGRP released in the pulp due to occlusal trauma stimulation play an important role in the inflammatory reaction. They interact with endothelial cells that cause the opening of intercellular pores to allow fluid release. Simultaneously, they provoke the release of histamine by granulocytes favoring plasma extravasation. They also activate the immune system by promoting chemotaxis and by recruiting and regulating other inflammatory cells of various myeloid and lymphoid lineages, dendritic cells, macrophages, epithelial progenitor cells, and DPSCs, thereby controlling the neurogenic inflammatory mechanism. Depending on the magnitude, duration of the stimulus, and state of the pulp tissue, a process of tissue repair may take place, or necrosis may develop if the pulp tolerance is exceeded (Nakashima et al).

In consequence, the pulp becomes locally inflamed in the presence of occlusal trauma due to the chemoattractant properties of SP and CGRP that attract cellular components to the cell-free zone in the tissue adjacent to the injury. Most of these cells have the same morphologic characteristics as fibroblasts and undifferentiated cells, and only a few are inflammatory-type cells. The formation of new capillaries induced by these neuropeptides is evident by the presence of erythrocytes produced by small blood extravasations (El Karim et al 2009, Kohara et al, Caviedes-Bucheli et al 2011a). In addition, CGRP acts synergistically with SP by increasing inflammatory mediators, compromising PBF and collateral circulation due to hypoxia caused by occlusal trauma. This initiates a compensation mechanism to correct the resulting damage, thereby avoiding vascular collapse, that will be greater depending on the stimulus intensity (Caviedes-Bucheli et al 2011b, Rechenberg et al). CGRP is involved in the formation of new vessels through the activation of the cAMP-PKA pathway, which is related to the stimulation of endothelial cells through its receptor CGRP-R1; this is an important mechanism for tissue repair through angiogenesis because it enhances the action of VEGF and the stimulation of fibroblasts and odontoblasts to form mineralized tissue as a defense mechanism (Arana-Chavez and Massa, Zheng et al, Caviedes-Bucheli et al 2017).

Simultaneous to these changes in the pulpal tissue, SP- and CGRP-mediated vascular degeneration also occurs. Arteriosclerotic changes in the blood vessels take place, thickening the tunica intima while the adventitia calcifies, decreasing blood flow to the pulpal tissue. The nerve fibers undergo a progressive mineralization of all their membranes, and fat deposits are also observed around the odontoblastic layer, the cell-rich zone, and the walls of the capillaries. The accumulation of interstitial fluid causes the presence of odontoblast vacuoles resulting from the separation of these cells from the dentin wall. There is a network of atrophic reticular fibers related to the high content of interstitial fluid and the decrease in the number of pulp cells. As aging progresses, the pulp continues to lose cellular content, while the number of collagen fibers continues to increase. The remaining odontoblasts are flat in shape and smaller in size. The number of capillaries and nerve fibers tends to decrease, and collagen is simultaneously produced to form a completely fibrous pulp (Morse, Goga et al, Cooper et al 2010, Lee et al 2013, Caviedes-Bucheli et al 2017).

Odontoblasts can be activated by SP and CGRP signaling; therefore, tertiary dentin production begins with a


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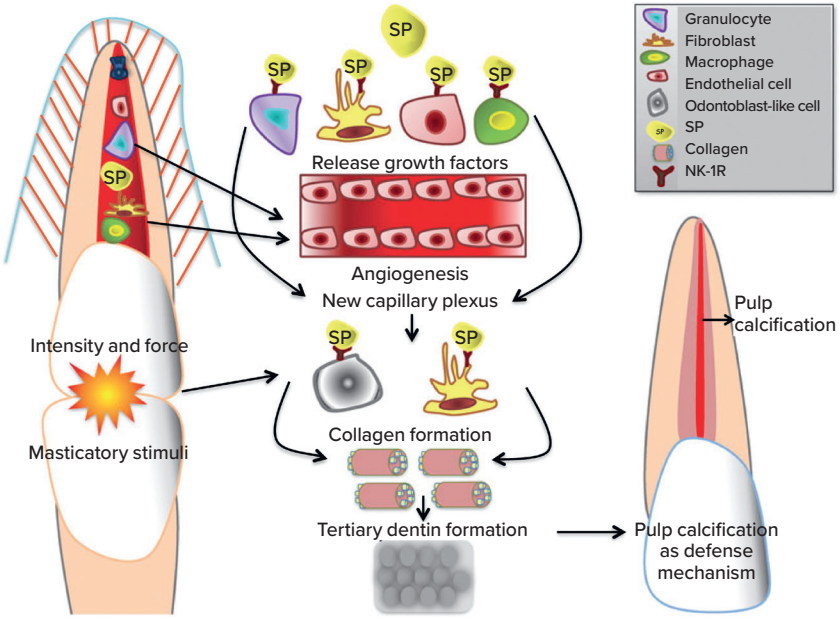


▶ **FIG 4-31** Response of human dental pulp to occlusal trauma and masticatory function. Angiogenic changes are mediated by SP through direct and indirect mechanisms where cells such as macrophages, granulocytes, endothelial cells, and fibroblasts release angiogenic factors that induce the formation of new blood vessels. Those vessels are necessary for the formation of collagen and tertiary dentin by redifferentiated fibroblasts, similar to the odontoblasts and odontoblast-like cells as a defense mechanism. (Reprinted from Caviedes-Bucheli et al 2017.)

mixture of direct deposits of mineral crystals within dentinal tubules that reduce their permeability in a short period of time, resulting in sclerotic dentin. To produce reactive tertiary dentin, the effect of the damaging stimulus needs to be much longer (Roberts-Clark and Smith, Arana-Chavez and Massa, Zhang et al 2011). After a longer period of time or after increasing the occlusal load, the reaction will be characterized by a marked cellular infiltration seen at the injury site and microabscess formation due to the compartmentalization capacity of the pulp. Polymorphonuclear and mononuclear leukocytes are also seen in the affected area. Odontoblast nuclei can be seen in the tubules due to increased fluid pressure and numerous vascular growths surrounding the intense cellular infiltration (Li et al 2011, Lee et al 2013). If the damage persists, the odontoblasts will no longer be able to defend the pulp, because they are postmitotic cells. At this stage, reparative dentin begins to form below the damaged tubular region, due to the action of different types of cells. There is an increase in the normal collagen portion of the pulp and a decrease in the cellular content. The noxious stimulus may destroy the underlying odontoblasts, and the pulp may require repopulation of the destroyed odontoblast layer based on differentiation of cells including perivascular cells, pulp fibroblasts, or undifferentiated cells formed by the odontoblast lineage but not previously exposed to the final epithelial influence during dentinogenesis (Tziafas, Cooper et al 2010, Smith et al).

The changes that take place in the connective tissue in response to both occlusal trauma and masticatory function are primarily mediated by fibroblasts, which express angiogenic factors that contribute to the formation of new blood vessels by direct mechanisms. These cells respond to mechanical stimuli due to a cascade of signals that come from the extracellular matrix and are mediated by receptors located on their surface, such as NK1 and CGRP-R1. Such signals also affect the morphology as well as the proliferation and differentiation of fibroblasts, which contain proteins called *kinases* that are sensitive to stress caused by the extracellular matrix. These proteins activate phosphorylation sites recognized by various molecular signals that modify the functioning of fibroblasts, inducing the formation of new blood vessels and mineralized tissue (Fig 4-31) (El Karim et al 2009, Krishnan and Davidovitch, Kohara et al).

If the stimulus persists after diagnosing the inflammatory process induced by occlusal trauma, the vascular neoformation cannot supply oxygen and nutrients to the tissues and therefore collapses, resulting in an accumulation of mediators, liquid, and plasma, because the vascular

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system is responsible for eliminating inflammatory mediators. This will cause a vicious cycle where the pulp that is confined in a low-adaptability environment will not be able to defend itself, and therefore pulpal necrosis will occur. This type of necrosis is characterized by the coagulation of intracellular proteins due to a low level of oxygen. It is important to note that only 7% of calcified pulps will develop pulpal necrosis (Holcomb and Gregory). This low percentage is probably due to the continuous formation of blood vessels induced by the constant release of SP that gives the tissue the ability to remain vital and viable (Killough et al 2009, Levin et al).

It can be concluded that occlusal trauma generates a neurogenic inflammatory response in human pulp, which can lead to tissue aging processes with the formation of mineralized tissue as a defense mechanism or to pulpal necrosis when the duration and magnitude of the stimulus exceeds the tolerance of the pulp (Caviedes-Bucheli et al 2011a, Caviedes-Bucheli et al 2017, Caviedes-Bucheli et al 2021).

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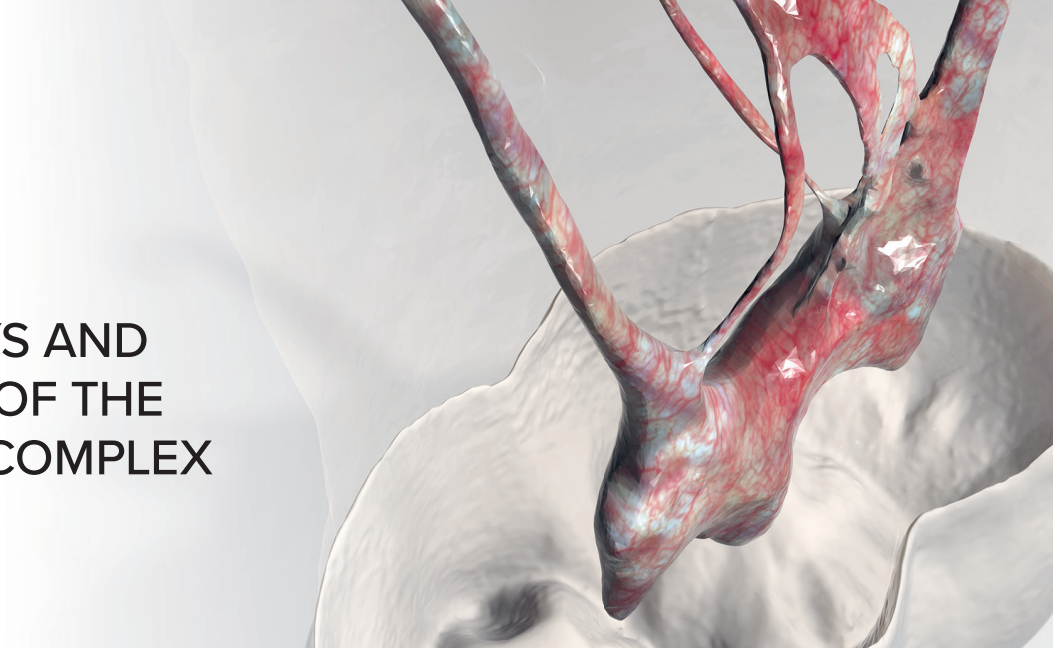

# 5

# PAIN PATHWAYS AND MECHANISMS OF THE DENTIN-PULP COMPLEX

Anibal Diogenes

Pain conditions are common in areas innervated by the trigeminal nerve, and this especially includes odontalgia (toothache) (Lipton et al, Riley and Gilbert). It is estimated that approximately 12% of the US population suffers from odontalgia, and it is the primary reason patients seek oral health care providers (Anderson and Thomas, Gilbert et al).

Although the subject of pain is of considerable importance to all health care providers, the simple reality is that many patients consider pain and dentistry to be synonymous. This association often leads to fear and anxiety, causing patients to delay treatment, ignore their initial symptoms, and contact oral health care providers only when the pain is unbearable (high intensity and chronic) (Armfield et al). This association is largely based on the patient's preexisting pain and previous untoward experiences following stimulation of dental nociceptors during treatment. Other factors, such as ethnicity, age, and sex, have all been correlated with different reported pain levels (Riley et al, Polycarpou et al). In addition, the media sometimes portrays dentists and oral health care providers negatively as professionals indifferent to their patients' pain, reinforcing anxiety toward dental treatment (Thibodeau and Mentasti).

In dental pain, activation of pulpal nociceptors is the initial step that ultimately results in the pain experience. However, pain is much more than activation of peripheral nociceptors; instead, pain represents an integration of nociceptive signals and emotional, cognitive, and affective components that make pain such a terrible experience (Price). Clinicians and researchers must never forget to appreciate this aspect of the pain experience.

Diagnosis and management of pain represent foundational skills necessary for the successful practice of dentistry and are especially critical when the patient with odontalgia is evaluated (Bender). Accordingly, this textbook provides an extensive review of pain mechanisms that are critical to the evaluation of the patient with odontalgia, including pulpal inflammation, neuroanatomy, and neurophysiology of the dental pulp, as well as effective pharmacologic and nonpharmacologic strategies for managing dental pain. The present chapter specifically contributes to the development of this foundational skill by reviewing peripheral and central nervous system mechanisms associated with the pain response following stimulation of the dentin-pulp complex. This knowledge base should help clinicians make more accurate diagnoses and design more effective pain-control strategies for their patients.

The awareness of pain involves three steps: *(1)* detection, *(2)* processing, and *(3)* perception (Fig 5-1). Detection is a function of the peripheral sensory (afferent) neuron; processing involves the selective activation of specific and related central nervous system pathways and is largely dependent on initial processing done within the medullary and spinal dorsal horns; and perception is the result of activity in more rostral brain regions such as the cerebral cortex. Together, the activities of these structures are responsible for the complex multidimensional aspect of pain as both a sensation and an emotion (Derbyshire et al). The clinician that diagnoses and treats odontalgia should consider the contributions provided by each of these steps because the pain experience is ultimately shaped by the combined activity of these various structures. The neuronal activity within these different pathways can change depending on the absence or presence of disease. Because most patients who seek endodontic therapy have disease, these modifying processes are active at the time of the clinical evaluation and may add to the challenges associated with diagnosis. This chapter reviews the peripheral and central pain mechanisms that should be considered when the clinician evaluates the symptomatic patient.

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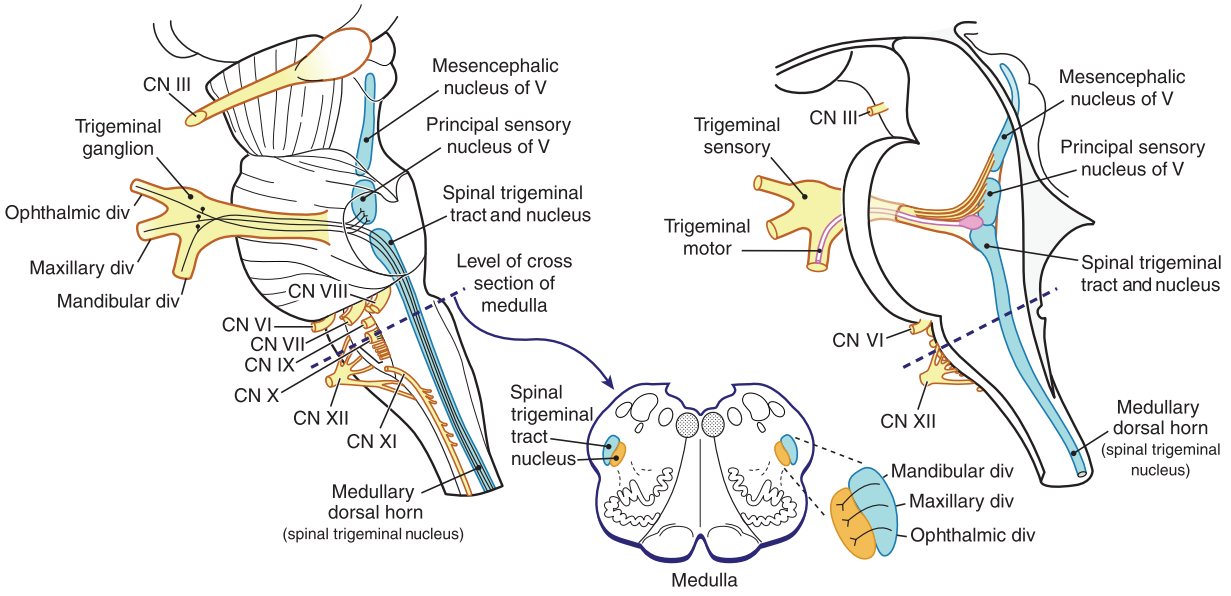


▲ **FIG 5-1** General steps in pain transmission in the orofacial region. Detection of noxious injury occurs via primary afferent nociceptors that travel in one of the three divisions (div) of the trigeminal nerve (ophthalmic, maxillary, and mandibular). (In certain chronic pain conditions, detection may also occur by other afferent fibers such as the Aβ-fibers.) Processing occurs primarily in the medullary dorsal horn. Nociceptive signaling may be increased by central mechanisms of hyperalgesia or allodynia. Nociceptive signaling may also be reduced by endogenous analgesic systems. The output from the medullary dorsal horn is conveyed predominantly along the trigeminothalamic tract. Perception occurs primarily in the cerebral cortex. Other sensory nerves are also responsible for additional craniofacial signaling (eg, cranial nerves [CN] VII, IX, and X as well as afferent fibers from the cervical spinal cord).

## Pathways Responsible for Detection, Processing, and Perception of Dental Pain

Odontogenic pain is usually the result of a noxious physical stimulus or the release of inflammatory mediators that stimulate receptors located on the terminal endings of nociceptive (pain-detecting) afferent C and Aδ nerve fibers (Woolf and Costigan, Willis, Hargreaves and Milam, Hargreaves 2001, Lund et al). Physical stimuli, via their effect on dentinal fluid flow, can activate the nociceptors that innervate dentinal tubules, leading to the perception of dentinal pain (Lund et al) (Fig 5-2). Inflammatory mediators, via activation of their respective receptors, can sensitize or depolarize the nociceptors that innervate pulp tissue. These topics are discussed in detail later in the chapter and elsewhere (Henry and Hargreaves). Experimental studies have shown that activation of nerves within the dental pulp by these physiologic (eg, thermal, mechanical, or chemical) stimuli results in a pure sensation of pain, whereas other studies using certain electrical stimuli can elicit a "prepain" sensation (Brown et al).

Interestingly, in addition to Aδ- and C-fibers, the dental pulp has been found to be innervated by myelinated low-threshold Aβ-fibers that normally convey responses to light touch and proprioception (Fried et al). Some of the confusion regarding the innervation pattern of the dental pulp arises from the observation that in human permanent teeth and experimental animals, the innervation appears to be largely myelinated (approximately 70%) (Gibbs et al) as it enters the tooth but progressively loses the myelin sheath as it approaches the coronal pulp and dentinal tubules, giving the appearance of unmyelinated C-fibers (Paik et al, Fried et al). Indeed, mouse molar teeth were found to be innervated by approximately 50 individual neurons (Emrick et al) composed primarily of fast-conducting nociceptors (presumably Aδ-fibers) but by approximately 25% fast-conducting fibers not traditionally characterized as nociceptors (Aβ-fibers). Thus, these results suggest a unique pattern of response to stimuli from the dental pulp that differs from other tissues. Because loss of dentition in the wild often results in death due to the inability to feed or, in the case of many animals, defend oneself (Jheon et al), the uniqueness of dental innervation is likely to be the result of evolutionary adaptation to respond to the subtle mechanical stimulation of fluid movement in dentinal tubules for the early detection of injury or potentially harmful stimuli.

The activation of the peripheral nociceptor produces a generator potential; if great enough, this depolarization will


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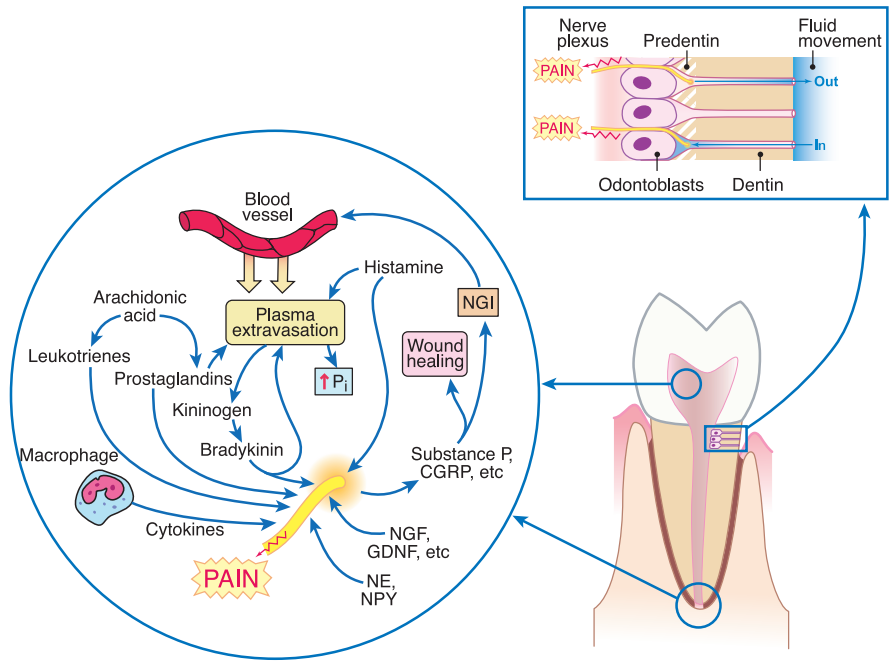
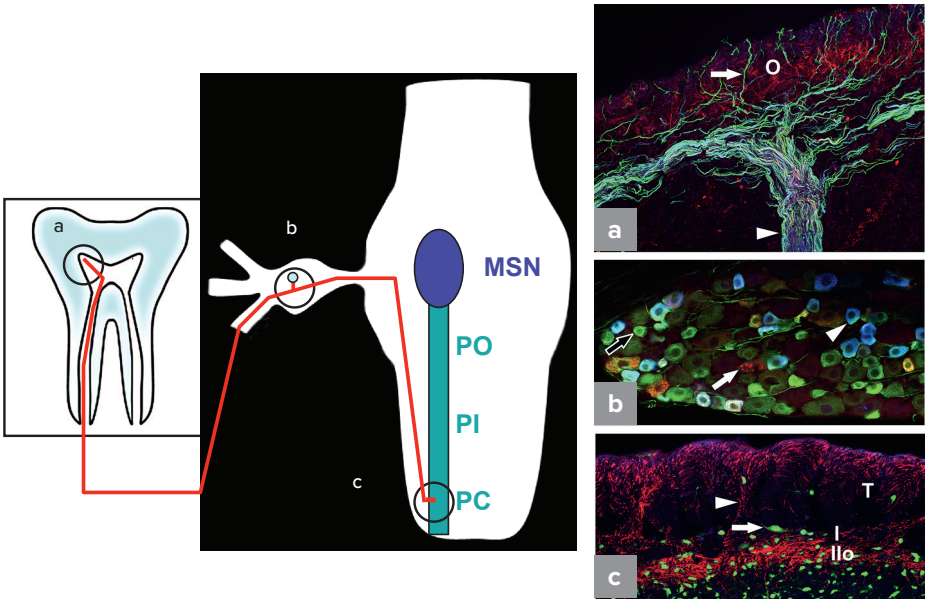



**▶ FIG 5-2** Two mechanisms for the peripheral stimulation of nociceptive nerve fibers in tooth pulp. *Acute dentinal pain:* According to the hydrodynamic theory, stimuli that cause fluid movement in exposed dentinal tubules result in the stimulation of nociceptive nerve fibers. *Pain with inflammation:* Inflammation is associated with the synthesis or release of mediators, including prostaglandins, bradykinin, substance P, and histamine (as well as other mediators not shown). The interrelationships of these inflammatory mediators form a positive feedback loop, allowing inflammation to persist far beyond cessation of the dental procedure. $P_i$ = intrapulpal pressure; NGI = neurogenic inflammation; CGRP = calcitonin gene-related peptide; NGF = nerve growth factor; GDNF = glial cell line–derived neurotrophic factor; NPY = neuropeptide Y; NE = norepinephrine.




**▶ FIG 5-3** Pathway and confocal micrographs of neuroanatomical structures responsible for the transmission of pulpal nociceptive stimuli within the trigeminal system. Peripheral nociceptive nerve fibers terminate as free nerve endings within the dental pulp *(a)* and arise from primary afferent cell bodies within the trigeminal ganglion *(b)*. The central processes of these primary afferent cell bodies pass into the brainstem and enter the trigeminal tract. These fibers exit the tract to terminate within the trigeminal sensory nucleus *(c)*, composed of the main sensory nucleus (MSN) and the spinal trigeminal nucleus. The trigeminal nucleus consists of pars oralis (PO), pars interpolaris (PI), and pars caudalis (PC). *(a)* Pulpal nerve fibers are seen within the pulp horn of a human specimen and are stained with antibodies against N52 *(green)*, PGP 9.5 *(blue)*, and TRPA1 *(red)*. A nerve fiber bundle *(arrowhead)* gives rise to an extensive arbor within the subodontoblastic plexus, with some fibers that enter and traverse *(arrow)* the odontoblastic layer (O). *(b)* Neuronal cell bodies are seen within the rat trigeminal ganglion and are stained with antibodies against peripherin *(green; black arrow)*, TRPV1 *(blue; arrowhead)*, and CGRP *(red; white arrow)*. Larger cell bodies lack staining, while the smaller cell bodies are stained individually or multiply with these antibodies used to identify nociceptors. *(c)* Intrinsic neuronal cell bodies are stained with NeuN *(green; arrow)*, and the central processes of CGRP-containing primary afferent fibers *(red)* are seen within a transverse section of the rat brainstem at the level of caudalis. The CGRP-containing primary afferent fibers are located in the trigeminal tract (T). Some of these fibers exit the tract *(arrowhead)* to enter and terminate within the superficial laminae I and II outer (o) zones of caudalis, where they form synapses with processes of intrinsic and descending neurons.

trigger a nerve impulse (action potential). The action potential is propagated along a peripheral trigeminal nerve to the primary afferent neuronal cell body located in the trigeminal ganglion and then into the central nervous system along the central process of this same neuron (Hargreaves and Milam, Sessle 1987, Sessle 2000, Sessle 2005) (Fig 5-3).

The central process of the primary afferent cell body enters the brainstem at the level of the pons by way of the

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trigeminal root entry zone and then enters the trigeminal tract. The trigeminal tract carries the primary afferent fiber to the trigeminal sensory nucleus located in the pons and medulla, where it then terminates. The most rostral portion of the trigeminal sensory nucleus is the main sensory nucleus, while the caudal portion is represented by the spinal trigeminal nucleus. The spinal trigeminal nucleus is further subdivided into the following subnuclei: pars oralis (most rostral), pars interpolaris, and pars caudalis (most caudal) (Brodal, Olszewski).

Animal studies have shown that primary afferent neurons that innervate dental pulp terminate in all of the different subnuclei located within the ipsilateral trigeminal sensory nucleus, including prominent projections to caudalis (Westrum et al). The projection to caudalis is expected because the tooth pulp is rich in pain fibers, and trigeminal primary afferent input to caudalis has been linked to facial pain. Trigeminal tractotomy at the level of pars caudalis in humans resulted in the loss of pain and temperature sensation over facial structures while preserving touch sensations (Sjöqvist), thus demonstrating a critical role for caudalis in facial pain perception. Even so, additional studies have shown that neurons located in more rostral nuclei can be activated by intraoral and perioral nociceptive stimuli, even after trigeminal tractotomy at lower levels, and together these studies demonstrate a broad projection of tooth pulp nociceptors throughout the trigeminal sensory nucleus (Woda). These projections form an important structural foundation for referred pain mechanisms that are of critical importance when the symptomatic patient is evaluated.

The trigeminal nucleus caudalis has been referred to as the *medullary dorsal horn* because it shares a similar laminar morphology with the spinal dorsal horn and its caudal extent merges with the dorsal horn at the upper cervical level (Gobel et al). Caudalis plays a critical role in the modulation and transmission of nociceptive information within the trigeminal sensory nucleus, and output to higher brain regions can be increased (ie, hyperalgesia), decreased (ie, analgesia), or misinterpreted (ie, referred pain) from the incoming activity (Sessle 2005).

As viewed from a functional perspective, the trigeminal sensory nuclei have five major components related to the processing of nociceptive signals: *(1)* central terminals of afferent fibers, *(2)* local circuit interneurons, *(3)* projection neurons, *(4)* terminals from descending neurons, and *(5)* glia. Because of the importance of caudalis to the modulation and transmission of pain in the trigeminal system, including that from the dental pulp, it will be used to illustrate these components.

The first components, primary afferent endings of C-fiber and Aδ-fiber nociceptors, enter caudalis via the trigeminal tract and terminate as axonal endings (Fig 5-4; see also Fig 5-1). These axonal endings form synapses with other neural components, which include local interneurons, projection neurons, and terminals from descending neurons (discussed later). Many of these nociceptors contain neuropeptides such as CGRP and substance P, and they terminate mainly in the superficial laminae (I and II) of the nucleus caudalis. These sensory fibers excite intrinsic neurons by releasing the excitatory amino acid glutamate along with neuropeptides (De Biasi and Rustioni).

The administration of receptor antagonists to glutamate and substance P, and to CGRP to a lesser extent, reduces hyperalgesia or nociceptive transmission in animal studies (Chiang et al, Ren et al, Dubner and Basbaum, Woolf). Evidence to date strongly indicates that antagonists to the glutamate receptor N-methyl-D-aspartate (NMDA) are particularly effective in reducing hyperalgesia in animal studies, as discussed later. Antagonists to the NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) classes of glutamate receptors are likely to serve as prototypes for future classes of analgesic drugs, and early clinical results in dental pain studies are promising (Woolf and Costigan, Gilron et al). Although animal studies indicate that antagonists to the neurokinin 1 class of substance P receptors block hyperalgesia, the results from clinical trials have been equivocal, and few studies have shown them to have significant effects on dental pain (Hill 2000, Dionne et al).

Local circuit interneurons are a second neural component of the caudalis. These neurons may act to modulate the excitability of both primary afferents and projection neurons or can act as an intermediary for the transfer of primary afferent input to projection neurons. Thus, in both situations they ultimately regulate the transmission of nociceptive signals from the primary afferent fibers to projection neurons (Willis, Sessle 2000, Light).

Interneurons comprise a diverse class of neurons whose morphologies relate to different electrical properties (Sedlacek et al). Depending on their neurotransmitter systems and anatomical connections, interneurons can enhance or suppress nociceptive processing (see Fig 5-4). Examples of inhibitory influences are provided by those that contain γ-aminobutyric acid (GABA; the main inhibitory neurotransmitter in the central nervous system) or opioids (Aicher et al) within synaptic vesicles located in their axon terminals and those that contact primary afferent axon terminals at axo-axonic inhibitory synapses to provide presynaptic inhibition of primary afferent input (Almond et al, Bae et al). Other influences are provided through postsynaptic mechanisms that involve the release of neurotransmitters from primary afferent terminals and receptors located on dendrites of second-order neurons (Todd and Spike). Therefore, the trigeminal nuclei are not simple relay stations between nociceptive signals from


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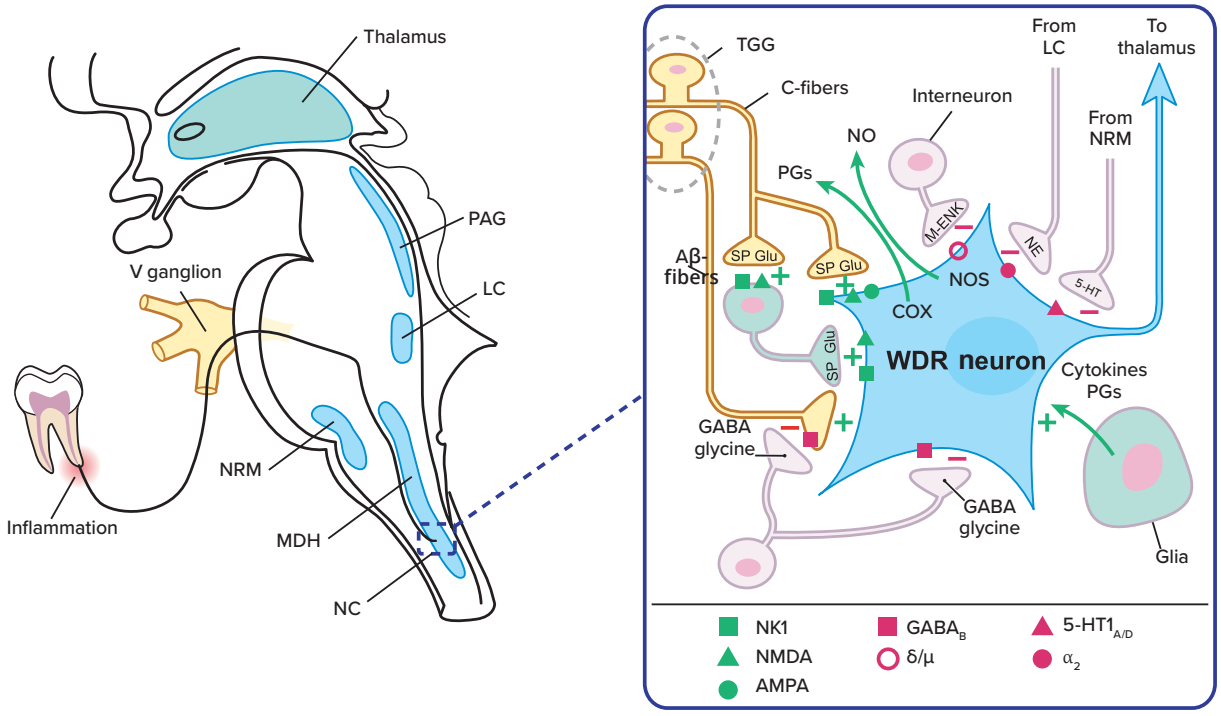
dental nociceptors to higher-order neurons (projection neurons). Instead, considerable pain-signal modulation occurs at these central sites.

The third neural component of caudalis is the projection neuron; the cell bodies of these neurons are within caudalis, and their axons comprise the output system for orofacial pain information because they project to other areas, including more rostral brain regions such as the thalamus. The projection to the thalamus involves the trigeminothalamic tract that crosses to the contralateral side in the brainstem before ending in different thalamic nuclei (as discussed later). Other caudalis neurons project to rostral regions of the trigeminal sensory nucleus, including oralis, where this intranuclear projection is especially important in mediating intraoral pain sensations (Woda, Sessle 2002).

Projection neurons can receive primary afferent input directly or indirectly. An example of indirect input involves primary afferent information that ends in lamina II and is processed by interneurons before subsequent activation of projection neurons located in lamina I. Again, the excitability of projection neurons can be enhanced or suppressed by synaptic interactions provided by other neural components located within caudalis, including its regulation by either excitatory or inhibitory interneurons or by the presence or absence of an inhibitory tone from descending pain pathways.

Three major classes of neurons within caudalis have been described, based on their physiologic responsiveness to different stimuli (Willis, Light). These include *(1)* the low-threshold mechanoreceptive type that is activated only by light tactile stimulation, *(2)* the nociceptive-specific (NS) type that is activated by high-threshold




▲ **FIG 5-4** Functional processing of nociceptive input in the nucleus caudalis (NC) of the medullary dorsal horn (MDH). In this example, activation of pulpal nociceptive C-fibers leads to the release of glutamate (Glu) and substance P (SP), which are conveyed across a synapse to a wide dynamic range (WDR) projection neuron. This projection neuron projects to the thalamus; the information is then relayed to the cortex. Glutamate binds and activates either NMDA or AMPA receptors, while SP binds and activates the neurokinin 1 (NK1) receptors. The sensory fibers can directly activate the WDR neuron or indirectly activate it via contacts with excitatory interneurons. Several signal transduction pathways have been implicated in modulating the responsiveness of projection neurons, including the protein kinase A (PKA) and protein kinase C (PKC) pathways. Projection neurons can themselves modulate nearby cells by synthesis and release of prostaglandins (PGs) via cyclooxygenase (COX) and nitric oxide (NO) via nitric oxide synthase (NOS). Glia can modulate nociceptive processing by release of cytokines (interleukin-1β and tumor necrosis factor) and PGs. Descending terminals of fibers originating in regions such as the nucleus raphe magnus (NRM) or locus coeruleus (LC) can release serotonin (5-HT) or norepinephrine (NE). Also depicted are the major proposed receptors for these neurotransmitters. Drugs that alter these receptors or neurotransmitters have potential as analgesics. 5-HT1$_{A/D}$ = 5-HT receptor; GABA$_B$ = GABA receptor; PAG = periaqueductal gray; TGG = trigeminal ganglion; M-ENK = met-enkephalin; δ/μ = δμ-opioid receptor; $\alpha_2$ = α2-adrenergic receptor.

nociceptive stimuli, and *(3)* the wide dynamic range (WDR) type that responds to both tactile and noxious stimuli.

A large body of evidence has focused on the role of the WDR and NS neurons in encoding orofacial pain and their contributions to the development of hyperalgesic and allodynic states (Woolf and Costigan, Hargreaves and Milam, Sessle 2000, Sessle 2005, Chiang et al, Dubner and Bennett, Maixner et al). These studies have shown that the peripheral receptive field of NS neurons is typically smaller than the receptive field of WDR neurons. The excitability and the receptive field of the WDR neurons both increase after inflammatory insults or nerve injury, and these changes correspond to a central sensitization that is the major mechanism underlying secondary hyperalgesia following these insults.

Evidence has accumulated about the receptors and signal transduction pathways in these projection neurons that lead to increased or reduced activity (Wilcox and Seybold, Yaksh and Malmberg). In particular, activation of intracellular protein kinases (eg, protein kinases A and C) and elevation of intracellular calcium levels are thought to facilitate responses to nociceptive input. These projection neurons are also thought to modulate the activity of nearby cells via release of prostaglandins and nitric oxide gas (Yaksh and Malmberg) (see Fig 5-4). The increased responsiveness involves an unmasking of normally inactive (latent) synaptic inputs and the activation of NMDA receptors on these neurons (Sessle 2005).

Another important physiologic feature of NS and WDR neurons relates to the issue of central convergence and involves the ability of these neurons to be excited by a wide variety of peripheral sources that include both superficial (cutaneous, mucosal) and deep (tooth pulp, muscle, jaw joint, dura cerebral blood vessels) structures. In addition, these same neurons can be activated by the application of sensory stimuli to structures that are innervated by cranial nerves VII, IX, and X and upper cervical afferents that project into the trigeminal sensory nucleus. This central convergence of peripheral inputs adds to the development of central sensitization following inflammatory insults and tissue or nerve injury. These physiologic properties should be considered when the clinician evaluates patients with trigeminal pain, especially those patients with preexisting insults, because they may contribute to the seemingly bizarre, referred pain patterns that may occur in trigeminal pain conditions such as toothache (Orbach and Hiesiger).

The fourth component of caudalis consists of the terminal endings of descending neurons (see Fig 5-4). These terminals modulate (inhibit or facilitate) the transmission of nociceptive information within the other caudalis neural components (Millan). One of the best-studied descending systems is the periaqueductal gray (PAG)–rostral ventromedial medulla (RVM) pathway (Fields). Stimulation of different neuronal populations within the PAG can lead to either an enhancement or inhibition of activity within nociceptive neurons located within caudalis and the dorsal horn.

Activation of this pathway involves endogenous opioid peptides (EOPs), especially within the PAG, and serotonergic inputs into caudalis and the dorsal horn from neurons located in the RVM. Another descending system involves the PAG–dorsolateral pontine tegmentum pathway that provides noradrenergic inputs. The analgesic action of some nontraditional (tricyclic antidepressants) and traditional (opioids) pain medications may involve activation of these descending modulatory pathways. The endogenous opioid peptides represent a critical element of this endogenous analgesic system. The EOPs are a family of peptides that possess many of the properties of exogenous opioids, such as morphine and codeine. The EOP family includes the enkephalins, dynorphins, and β-endorphin–related peptides.

EOPs are found at several levels of the pain-suppression system. This fact underlies the analgesic efficacy of endogenous and exogenous opioids because their administration conceivably activates opioid receptors located at all levels of the neuroaxis. The EOPs are probably released during dental procedures because blockade of the actions of endogenous opioids by administration of the antagonist naloxone can significantly increase the perception of dental pain (Hargreaves et al 1986, Gracely et al, Levine et al). In animal studies, application of mustard oil to the dental pulp produces a profound increase in muscle activity, and this effect is enhanced by naloxone, which prolongs the activity after mustard oil application, an effect not observed when naloxone was administered after the vehicle (mineral oil) exposure to the dental pulp (Sunakawa et al) (Fig 5-5). In humans, administration of naloxone significantly increases pain perception during dental procedures (Hargreaves et al 1986) (Fig 5-6). Collectively, these studies indicate that orofacial pain activates an endogenous analgesic system involving the release of opioids and that blockade of this inhibitory tone by naloxone increases pain perception.

The endogenous cannabinoid system represents another modulatory system that can inhibit the central terminals of C-fibers; hypoactivity of this system and disinhibition of the central terminals may mediate some forms of chronic pain (Richardson et al 1998a, Richardson et al 1998b, Cravatt and Lichtman). Cannabinoids may have profound effects for modulating pain because there are about 10 times more cannabinoid receptors than opioid receptors in the central nervous system. Additional studies have demonstrated cannabinoid receptors on sensory neurons and dental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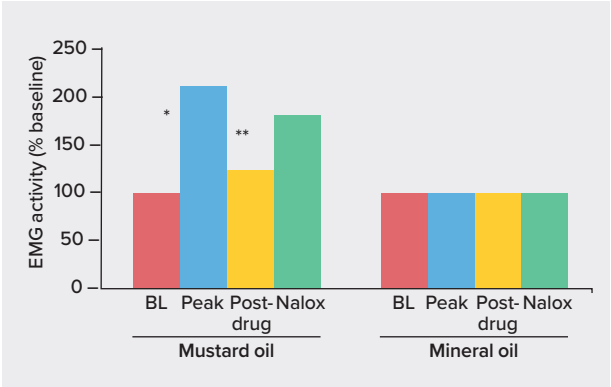
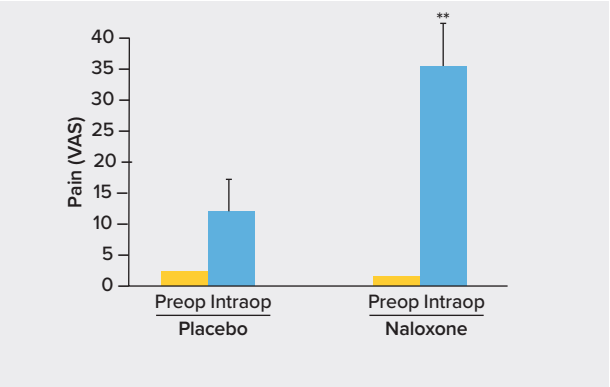



▲ **FIG 5-5** Effect of stimulation of dental pulp nociceptors on masseter muscle electromyographic (EMG) activity in anesthetized rats. Either mustard oil or its vehicle was injected into maxillary first molars. EMG data are presented as a percentage of baseline (BL) activity. Data were collected 30 minutes after drug administration (postdrug), and then both groups of animals were administered naloxone (Nalox), an opiate receptor antagonist (1.2 mg/kg, intravenously). *$P < .05$ (ANOVA) versus baseline; **$P < .05$ versus mustard oil. (Data from Sunakawa et al.)




▲ **FIG 5-6** Effect of naloxone administration versus placebo on intraoperative pain levels in dental patients undergoing surgical removal of impacted third molars. Patients were anesthetized with local anesthetic and, 10 minutes into surgery, were given an intravenous injection of either naloxone (10 mg) or placebo on a double-blinded randomized basis. **$P < .01$ (ANOVA). VAS = visual analog scale (0 to 100). (Data from Hargreaves et al 1986.)

pulp, where they may act to inhibit peripheral terminals of unmyelinated nociceptors (Hohmann and Herkenham, Wurm et al, Richardson et al 1998b). Further, the prominent expression of cannabinoid receptors within the descending inhibitory pathways appears to underlie the efficacy of acetaminophen (paracetamol) for treating dental pain. This effect is due to the formation of AM404, a known metabolite of acetaminophen that is preferentially formed in the central nervous system and acts as a ligand for the cannabinoid receptor 1 (CB1) and as an inhibitor of the endogenous cannabinoid reuptake system, thus leading to an increase in the endogenous cannabinoid inhibitory tone (Högestätt et al, Mallet et al). Therefore, the endogenous cannabinoid system represents a major pharmacologic target for the treatment of odontogenic pain.

Glia constitute the fifth component of caudalis involved in pain processing. Although glia were initially viewed as merely supportive cells, later studies strongly implicated the active participation of microglia and astrocytes in the processing of nociceptive input within caudalis and the dorsal horn (Watkins et al 2001, Tsuda et al, Sessle 2007, Salter). Glia respond to nociceptive input and release substances such as proinflammatory cytokines (interleukin-1 and tumor necrosis factor α), excitatory amino acids, adenosine triphosphate, nitric oxide, and prostaglandins and express ion channels and receptors for various neuromodulators and neurotransmitters that can regulate the release of these substances. Indeed, the release of inflammatory mediators such as prostaglandin E2 from the microglia in the hyperalgesic state has been shown to result from long-term opioid medication usage (Hayes and Painter). In this paradoxical state, opioid medications do not promote analgesia; instead their long-term usage results in activation of the toll-like receptor 4 (TLR-4) and the release of inflammatory mediators sensitizing spinal neurons, underlying the mechanism of this form of chronic pain, now recognized as *opioid-induced hyperalgesia* (OIH) (Watkins et al 2009). Although some of the signals responsible for the activation of glia are unknown, there is increasing evidence for the participation of endogenous damage-associated molecular patterns (DAMPs) in the activation of glia within caudalis, contributing to pain after both inflammatory and nerve lesion insults in the trigeminal system (Piao et al, Guo et al, Xie et al).

Some of the neurons within caudalis that are activated by nociceptive stimuli project to higher brain centers (Dostrovsky 2005). Much less is known about this topic than the local processing of nociceptive information within caudalis, but animal and human studies show projections into lateral and medial nuclei within the thalamus (Craig). This segregation is important because subsequent projections from these thalamic nuclei are thought to preferentially subserve the different sensory and affective aspects of pain (discussed later in this chapter). The lateral group

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that receives projection from caudalis neurons includes the posterior part of the ventromedial nucleus, the ventroposterior inferior nucleus, and the ventral posteromedial nucleus (part of the ventrobasal complex). The medial group includes the medial dorsal nucleus and the central lateral nucleus.

Additionally, there are projections to brainstem structures that include catecholamine cell groups, parabrachial nucleus, PAG, reticular formation, and superior colliculus. The projection to the parabrachial nucleus is significant and provides important projections to the amygdala, hypothalamus, and medial thalamus (Li et al). The projections to the amygdala and hypothalamus are noteworthy because these both project back to the PAG, where these inputs are considered critical for the control of the PAG descending system that was discussed earlier (Heinricher).

The perception of pain appears to occur within a variety of cerebral structures, and together this system represents the pain neuromatrix (Melzack). This concept is helpful to evaluate the various sensory-discriminative and affective-motivational aspects that together create the multidimensional pain experience. This matrix involves parallel processing through a continuation of the lateral and medial pathways seen at the thalamic level. Much of this information has been gained through functional imaging studies of a variety of human pain states, including trigeminal pain conditions and tooth pain (Borsook et al, Jantsch et al).

Prominent projections to the lateral system include those to the primary and secondary somatosensory cortices involved in encoding stimulus duration and localization. The medial system projections include anterior cingulate and dorsolateral prefrontal cortices, insula, and the amygdala. The anterior cingulate cortex is commonly activated and has been linked to the affective component of pain, whereas the amygdala is correlated with the processing of fear. Studies have also shown the activation of subcortical structures such as the hypothalamus and the PAG that are important components of the descending modulatory pathway (Borsook et al, Jantsch et al).

Key findings regarding the functional pain neuromatrix are that the psychologic context associated with the stimulus can entirely change neuronal activity, and just the anticipation of a painful stimulus can activate the matrix (Porro et al). Conversely, it has been shown that if a high-intensity painful stimulus is expected by patients in a predictable manner, less activation of hippocampus is observed compared to an unexpected stimulus, even if it is of lower intensity (Lin et al). Thus, the unpredictability of pain experiences puts patients at risk for heightened painful experiences. Altogether, these findings have significant implications for the way patients should be treated, including the need to avoid "surprise" painful experiences as they will shape patients' responses to subsequent dental treatment visits and may result in what is known as "pain catastrophizing."

Activity within the matrix can shift pathways, and the dependence on any one structure or pathway is plastic. The complex neuronal plasticity in painful states may result in short- and long-term changes in the pain-processing pathways. For example, trigeminal pain can be modulated at various locations within the pain system, including the trigeminal nucleus and other brainstem locations, subcortical sites, and cortical sites, and painful conditions can lead to significant reorganization within any of these sites.

These are all important considerations when the patient in pain is evaluated because activity within the entire pain neuromatrix, and not just activity within the tooth itself, produces the pain associated with toothache, which is an experience that integrates all the inputs discussed above with the patient's own fears, expectations, and coping mechanisms. Nonetheless, events within the dentin-pulp complex can be the first step in the activation of this trigeminal pain pathway.

## Mechanisms of Dental Pain Caused by Inflammation

Inflammation is common in the diseased dental pulp and leads to the activation of pulpal nociceptors and odontogenic pain. Although increased activity in fast-conducting fibers (such as Aδ-fibers within dentinal tubules) is present in pulpitis and clinically manifests as exaggerated fast responses to thermal changes (eg, heightened responses to cold test), much of the odontogenic pain is thought to arise from the activation of unmyelinated (C-fiber) nociceptors. This hypothesis is supported by the distribution of C-fibers in dental pulp, their responsiveness to inflammatory mediators, and the strikingly similar perceptual qualities of pain associated with C-fiber activation and pulpitis (ie, dull, aching pain) (Närhi, Ahlquist and Franzen 1994a, Mumford and Bowsher 1975). In a clinical study, the dental pulp of human volunteers was exposed to either inorganic ions or inflammatory mediators, with marked differences in the quality of pain felt (Ahlquist et al 1985). The application of histamine and bradykinin primarily produced reports of dull, aching pain (Fig 5-7). Similar results have been observed in electrophysiologic studies evaluating the responsiveness of Aδ and C afferent neurons to stimuli applied to deep cavity preparations (Närhi et al 1992a) (Fig 5-8). The Aδ-fibers respond to stimulation of dentinal tubules (eg, air blast or cold test), whereas pulpal C-fibers respond to bradykinin or capsaicin (Närhi et al 1992a, Närhi et al 1992b). Collectively,


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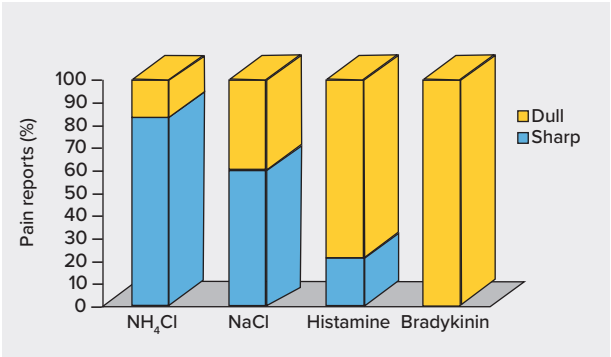
these and other studies have implicated pulpal Aδ-fibers in mediating dentinal sensitivity and pulpal afferent C-fibers in mediating pulpal inflammation (see Fig 5-2).

The dental pulp is one of the most heavily innervated and vascularized tissues in the human body and is capable of exuberant inflammatory responses. Microbial infections are the most prevalent etiology of pulpal inflammation, followed by traumatic injuries. The inflammatory reaction evoked by microorganisms is a complex response that involves recognition of antigenic molecular patterns and the subsequent coordinated release of multiple classes of inflammatory mediators that display distinct profiles of substance concentration over time (Hargreaves and Hutter).

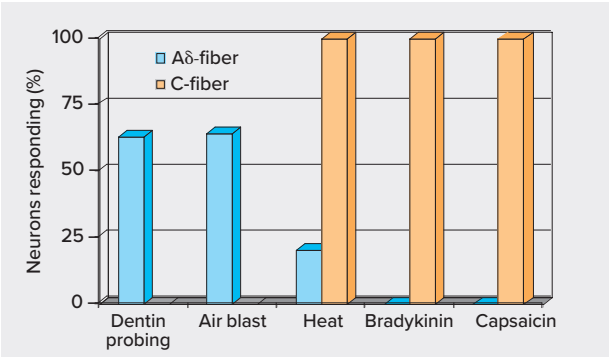
Lipopolysaccharide (LPS; also known as *endotoxin*) is one of the most prominent microbial antigens in dental infections due to the high representation of gram-negative bacteria (Hashioka et al, Siqueira et al). This highly potent activator of the innate immune response is recognized by a specific receptor (TLR-4) expressed on the plasma membrane of cells of the innate immune response (Janssens and Beyaert, Miyake). Interestingly, TLR-4 is also expressed in dental nociceptors (Wadachi and Hargreaves), and neuronal recognition of this bacterial component activates and sensitizes these dental pain–sensing fibers, leading to increased nociceptive signals and release of proinflammatory neuropeptides (Diogenes et al 2011, Ferraz et al) (Fig 5-9).

Given that nociceptors can extend up to 0.16 mm into dentinal tubules (Byers 1980) and that LPSs diffuse apically, reaching the pulp faster than bacteria (Khabbaz et al), it is very likely that nociceptors in the dentinal tubules will be activated, alerting the host to an incoming bacterial infection. In addition, vasoactive peptides such as CGRP and substance P will initiate and amplify the pulpal inflammatory reaction (neurogenic inflammation), acting in conjunction with cytokines released from dendritic cells and resident immune cells. These early symptoms, when acknowledged and treated, favor the maintenance of pulpal vitality. Therefore, primary afferent terminals in the dental pulp and periradicular tissues can directly "sense" the bacterial presence by the activation of TLR-4 and subsequent activation of these nociceptors, leading to an alerting pain signal.

Importantly, clinical levels of LPS have been directly correlated to pain symptoms in patients in multiple studies (Gabrielli et al, Bronzato et al, Gomes et al 2009, Martinho and Gomes). Also, LPS appears to be resistant to chemomechanical preparations during root canal therapies, with considerable detectable levels of LPS still present. This is an important observation because high levels of LPS are solubilized in the form of endotoxin upon bacterial death and lysis, so when it remains in the root canal structure, it can potentially activate nearby nociceptors through the direct activation of TLR-4 or indirectly through the release of inflammatory mediators that in turn activate and sensitize nociceptors. It is believed that residual LPS or other microbial antigens could perpetuate activation of nociceptors and inflammation even in the absence of active microbial infection. It is noteworthy that high levels of lipoteichoic acid (LTA), derived from gram-positive




▲ **FIG 5-7** Effect of application of inorganic ions or inflammatory mediators to exposed human dental pulp. Volunteers received mepivacaine anesthesia and had their pulp exposed via a Class V cavity preparation. After the patients had recovered from anesthesia, the cavity preparations (N = 16) were dried, and test solutions of ammonium chloride ($NH_4Cl$; 0.77 mol/L), sodium chloride (NaCl; 0.77 mol/L), histamine (10 mg/mL), or bradykinin (10 µg/mL) were placed on the surface of the pulp. The stimulus period was less than 3 minutes, and the interstimulus interval was 5 minutes. The quality of the evoked pain was recorded after application of the test agent. (Data from Ahlquist et al 1985.)




▲ **FIG 5-8** Responsiveness of pulpal Aδ afferent neurons and pulpal C afferent neurons to different stimuli applied to deep dentinal cavity preparations in anesthetized cats. (Data from Närhi et al 1992a, Närhi et al 1992b.)

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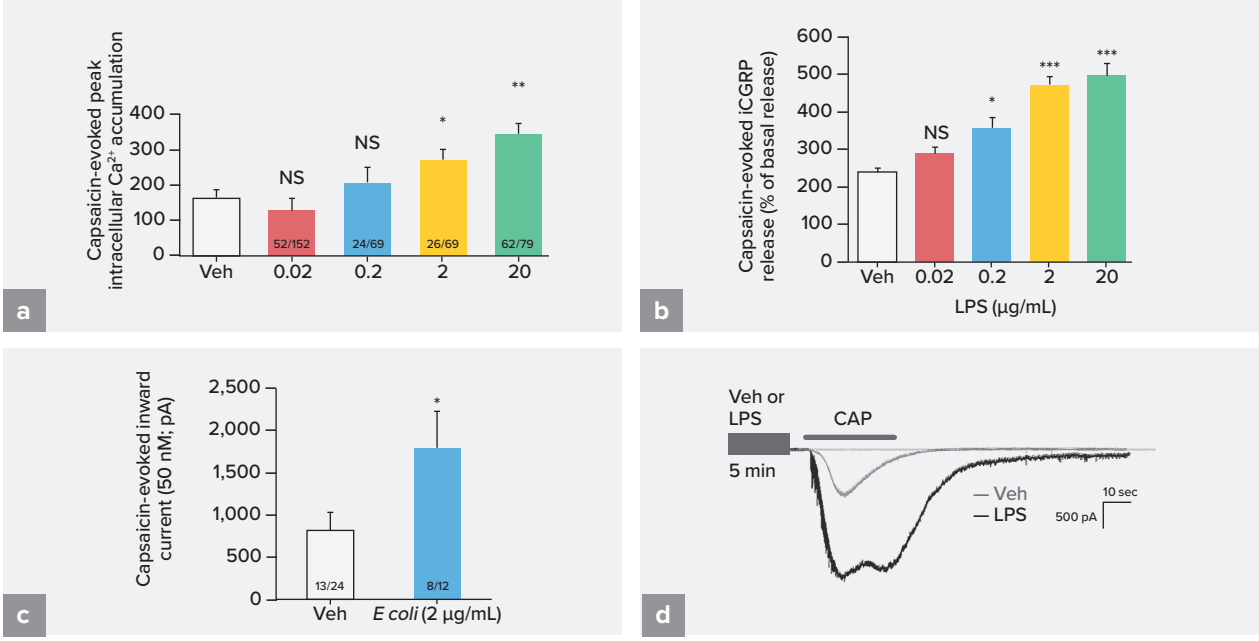


▲ **FIG 5-9** Lipopolysaccharide (LPS) sensitizes transient receptor potential vanilloid type 1 (TRPV1) responses in a concentration-dependent manner. *(a)* Pretreatment of trigeminal neurons with LPS evoked a concentration-dependent sensitization of a subsequent capsaicin-evoked accumulation of intracellular calcium. *(b)* Pretreatment of trigeminal neurons with LPS evoked a concentration-dependent sensitization of capsaicin-evoked immunoreactive CGRP (iCGRP) release. *(c)* Pretreatment of trigeminal neurons with LPS (2 µg/mL) led to the sensitization of a subsequent capsaicin-evoked inward current. *(d)* Representative traces of LPS sensitization of capsaicin-evoked currents. Veh = vehicle; NS = not significant; CAP = capsaicin. Data are presented as mean ± standard error. *$P < .05$; **$P < .01$; ***$P < .001$. (Reprinted with permission from Diogenes et al 2011.)

bacteria, are similarly present in infected root canals (Gomes et al 2022) and can also activate and sensitize nociceptors through the activation of toll-like receptor 2 (TLR-2), which has been demonstrated to be expressed in a subpopulation of neurons (Wang et al, Lima et al).

As mentioned previously, LPS-activated immune cells release a cascade of inflammatory mediators (Kawai and Akira). If any given mediator reaches a concentration in the inflamed tissue sufficient to activate specific receptors expressed in nociceptors, these nociceptive neurons could become activated (ie, the membrane would be depolarized and the signal would be conducted to the central nervous system or sensitized) (see Fig 5-2). A sensitized nociceptor displays spontaneous depolarization, reduced threshold for depolarization, and increased after-discharges to suprathreshold stimuli. Therefore, dental nociceptors can be activated directly by bacteria and their byproducts and indirectly by the microorganism-mediated release of inflammatory mediators, which in turn may activate and/or sensitize nociceptors.

Some inflammatory mediators activate these terminals (eg, bradykinin, LPS), while some potentiate the effects of other inflammatory mediators (eg, prostaglandins). For example, prostaglandin E2 substantially increases the stimulatory effect of bradykinin (Goodis et al) (Fig 5-10). Therefore, the combination of mediators ("inflammatory soup") present is probably more important than the presence of any one mediator in determining the physiologic response to inflammation.

Other inflammatory mediators may produce persistent effects. For example, nerve growth factor is expressed in inflamed dental pulp, and its concentration can increase up to eightfold in the inflamed dental pulp (Wheeler et al). It has been demonstrated that a single injection of nerve growth factor in humans can evoke pain and allodynia that lasts up to 1 month (Byers and Närhi, Petty et al). This process of nociceptor sensitization has important clinical implications because it contributes to the altered pain states of hyperalgesia and allodynia (Woolf and Costigan, Lewin et al). This long-lasting effect of nerve growth factor and other mediators appears to be related to changes in gene expression leading to a different cellular physiology (Diogenes et al 2007). This change in neuronal phenotype (neuronal plasticity) has profound, long-lasting effects on the detection and processing of nociceptive signals.

The overall neuronal plasticity that results from persistent inflammation could lead to significant changes in the nociceptive pathways. For example, the expression of the voltage-gated sodium channels Nav1.7, Nav1.8, and Nav1.9 has been found to be dramatically increased in the inflamed dental pulp (Wells et al, Luo et al, Warren et al, Henry et al 2009) (Fig 5-11). In addition, inflammation


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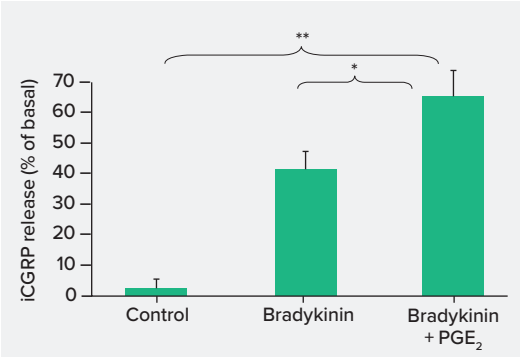
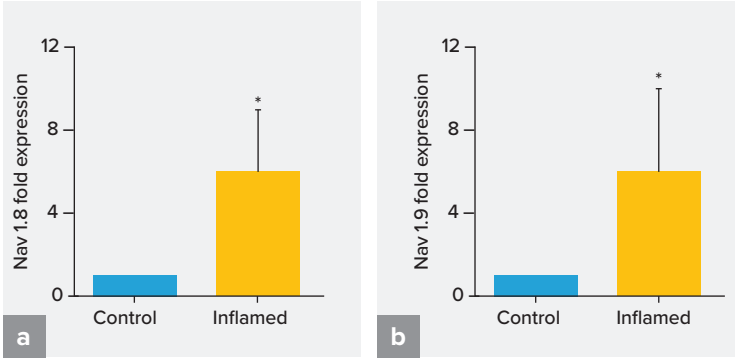



▲ **FIG 5-10** Effect of bradykinin alone and with prostaglandin E2 ($PGE_2$) on the release of immunoreactive CGRP (iCGRP) from isolated superfused bovine dental pulp slices. Tissue was pretreated with bradykinin alone or with bradykinin and $PGE_2$, and the released levels of iCGRP were measured by radioimmunoassay. *$P < .05$ (ANOVA); **$P < .01$ (ANOVA). (Data from Goodis et al.)




▲ **FIG 5-11** Expression of the sodium channels Nav1.8 *(a)* and Nav1.9 *(b)* is increased in the inflamed dental pulp. Data are expressed as fold change over control (uninflamed dental pulp). *$P < .05$. (Data from Wells et al and Warren et al.)

changes the expression pattern of these channels to axonal sites different than the typical nodes of Ranvier (Henry et al 2006). These changes in both quantity (increased expression) and quality (expression site) have a significant effect on the transmission of action potentials generated in the innervated target tissues (dental pulp and periradicular region).

Another important example of this effect is the long-term effect of endotoxin on neuronal phenotype. As mentioned previously, pulpal nociceptors carry the receptors for LPS (endotoxin), and these neurons are activated and become acutely sensitized as a result of the identification of endotoxin (Diogenes et al 2011). However, it has been demonstrated that LPS triggers genomic changes in the dental pulp nociceptors, leading to upregulation of the expression of the transient receptor potential vanilloid type 1 (TRPV1) channel, a crucial inflammatory pain-signaling molecule (Caterina et al 1999, Chung et al). Therefore, infection and inflammation could lead to long-lasting changes that could persist after the removal of the inflammatory stimulus and could be involved in persistent painful states.

Signal transduction studies have revealed some of the fundamental mechanisms mediating the actions of inflammatory mediators and drugs on sensory neurons (Wilcox and Seybold, Yaksh and Malmberg, Julius and Basbaum, Dray, Southall and Vasko). For example, inflammatory mediators (such as bradykinin) whose receptors couple to the $G_q$ guanosine triphosphate binding protein, which leads to activation of the protein kinase C pathway of secondary messengers, activate nociceptors. On the other hand, inflammatory mediators (such as prostaglandins) whose receptors couple to the $G_s$ guanosine triphosphate binding protein, which leads to activation of the protein kinase A pathway of secondary messengers, sensitize nociceptors. Finally, drugs (such as opiates, cannabinoids, and adrenergic agonists) that activate receptors coupled to the $G_i$ guanosine triphosphate protein tend to be analgesics. This is a simplification of a very active research front that is likely to lead to fundamental advances in the knowledge of pain mechanisms and the development of new classes of analgesic drugs.

## Allodynia and hyperalgesia

The pain system can undergo dramatic changes in response to certain peripheral stimuli, leading to the development of allodynia and hyperalgesia (Willis, Sessle 2000, Dubner and Basbaum, Woolf, Dubner and Ruda, Hargreaves and Keiser). These changes do not necessarily take weeks to develop but under certain conditions can occur within a few seconds or minutes after an appropriate stimulus.

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**TABLE 5-1 Evaluation of hyperalgesia and allodynia in endodontic diagnostic tests**

| Sign of hyperalgesia or allodynia | Related diagnostic test or symptom |
|---|---|
| Spontaneous pain | Spontaneous pain |
| Reduced pain threshold | Percussion test; palpation test; throbbing pain |
| Increased response to painful stimuli | Increased response to pulp test (electrical or thermal) |

Reprinted with permission from Hargreaves et al 1994.

For example, extensive hyperalgesia and allodynia occur in humans a few minutes after stimulation of cutaneous C nociceptors by injection of capsaicin (LaMotte et al). These two different pain entities are detected by an increased response to a painful stimulation of the capsaicin site (hyperalgesia) and by the sensation of pain when the skin near the injection site is gently stroked with a cotton swab (allodynia).

*Hyperalgesia* is defined as an increase in the perceived magnitude of a painful stimulus, and *allodynia* is defined as a reduction in pain threshold so that previously nonnoxious stimuli are perceived as painful. Many people have experienced these altered pain states; common examples include sunburn or thermal injury. In sunburn, the pain experienced when a T-shirt is worn is allodynia (ie, reduced pain threshold), whereas the increased pain responsiveness experienced when someone slaps the burned skin is hyperalgesia (ie, increased pain perception).

Allodynia or hyperalgesia can occur during inflammation of pulpal or periradicular tissue. In fact, clinicians often rely on clinical testing and the patient's symptoms to detect the presence of hyperalgesia and allodynia (Hargreaves et al 1994) (Table 5-1). For example, thermal hyperalgesia is detected in the clinical setting when an exacerbated response is seen to the cold vitality test using carbon dioxide or 1,1,1,2-tetrafluoroethane (eg, Endo-Ice, Hygenic). On the other hand, a painful response to the gentle tapping of a tooth with the mirror handle (or pain on mastication) is an indication of the presence of mechanical allodynia. Under normal conditions, this innocuous stimulation does not elicit pain. However, under conditions of acute or chronic inflammation, the mechanical pain threshold is reduced to the point at which tapping with a mirror handle is perceived as tender or painful.

A quantitative method based on the use of a digital force transducer has been developed as an alternative to the subjective tapping of teeth with the mirror handle. This novel method is capable of quantifying the severity of mechanical allodynia (Khan et al 2007a, Khan et al 2007b). It has been found that 57% of patients diagnosed with irreversible pulpitis have significant mechanical allodynia (Owatz et al) and that the mechanical pain threshold was reduced by 77% (Khan et al 2007b). This quantitative tool revealed the magnitude of mechanical allodynia in patients with various pulpal and periradicular diagnoses. The greatest reduction in mechanical pain thresholds were observed in patients diagnosed with irreversible pulpitis and symptomatic apical periodontitis (Alelyani et al). Interestingly, mechanical allodynia was detected even in patients with normal responses to clinical subjective diagnostic tests (ie, with diagnosis of asymptomatic irreversible pulpitis or asymptomatic apical periodontitis) (Alelyani et al) (Fig 5-12). Therefore, the systematic evaluation of allodynia or hyperalgesia represents the biologic rationale for endodontic diagnostic tests and provides unique information on the relative concentration of inflammatory mediators in the periradicular tissues (ie, degree of inflammation).

Similarly, studies in cats have shown that pulpal inflammation lowers the mechanical threshold of pulpal fibers to the level at which increases in systolic blood pressure can activate pulpal neurons. The synchronous firing of pulpal fibers in response to the heartbeat is thought to mediate the "throbbing" pain of pulpitis (Närhi, Byers and Närhi). Additional studies have shown that the thermal threshold of nociceptors can be reduced to the point at which normal physiologic temperature (ie, 37°C) can activate these peripheral neurons (Chuang et al, Diogenes et al 2006). This may explain why some patients use ice water to relieve pain from severe, irreversible pulpitis because the neurons would be expected to stop firing when the tissue is cooled. Accordingly, the mechanisms and management of allodynia and hyperalgesia are important issues in managing dental pain.

### Peripheral mechanisms of allodynia and hyperalgesia

Hyperalgesia occurs not only in inflammation and tissue injury (primary hyperalgesia) but also in adjacent areas


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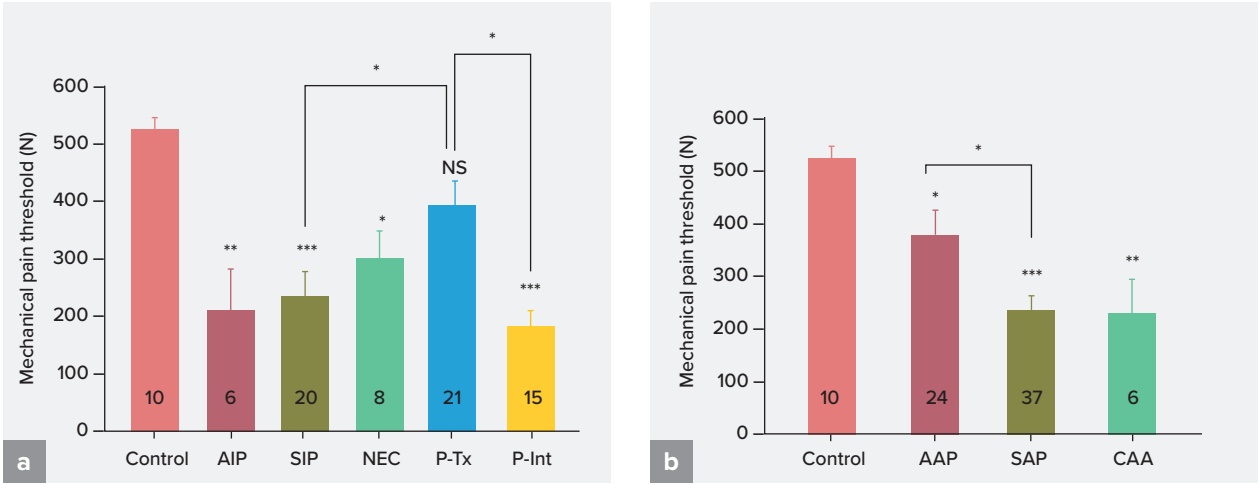


▲ **FIG 5-12** Bite withdrawal threshold quantification in patients with different pulpal and periradicular diagnoses. *(a)* Teeth diagnosed with asymptomatic irreversible pulpitis (AIP), symptomatic irreversible pulpitis (SIP), pulp necrosis (NEC), and previously initiated therapy (P-Int) had marked mechanical allodynia seen as a reduction in bite withdrawal threshold. *(b)* Teeth diagnosed with asymptomatic apical periodontitis (AAP), symptomatic apical periodontitis (SAP), and chronic apical abscess (CAA) also had marked mechanical allodynia. One-way ANOVA with Bonferroni's post hoc test; **P* < .05; ***P* < .01; ****P* < .001. NS = not statistically significant. Numbers in bars denote sample size for each group. (Reprinted with permission from Alelyani et al.)

(secondary hyperalgesia) (Lewis). Peripheral mechanisms are responsible for pain responses within the zone of primary hyperalgesia, whereas central mechanisms are responsible for the development of secondary hyperalgesia (Woolf and Costigan, Willis, Hargreaves and Milam, Hargreaves 2001, Sessle 2000, Dubner and Bennett).

Although both hyperalgesia to heat and hyperalgesia to mechanical stimuli are observed in primary hyperalgesia, only mechanical hyperalgesia is seen in the zone of secondary hyperalgesia (Raja et al). Moreover, the nociceptors that become sensitized within the primary zone of hyperalgesia produced by heat injury include a class of A-fiber nociceptors that are responsible for heat hyperalgesia, while mechanical hyperalgesia within this area is produced by the sensitization of a class of nociceptors that are typically insensitive to mechanical stimuli but respond to this stimulus after inflammation. These are called *silent nociceptors* and exist in a variety of tissue types (Campbell and Meyer). The zone of secondary hyperalgesia that results from pulpal inflammation has not been evaluated but most likely has clinical relevance in relation to patient symptoms and the results of certain clinical testing procedures.

Several mechanisms have been proposed to contribute to peripheral hyperalgesia (Box 5-1). As mentioned previously, the concentration and composition of various inflammatory mediators are important and can lead to the activation or sensitization of nociceptors (see Fig 5-2). The concentration of the inflammatory mediator is important because tissue levels must be sufficiently high to permit binding and activation of the receptor. For example, prostaglandin E2 levels are more than 100-fold greater in pulp samples collected from teeth with irreversible pulpitis than they are in normal control teeth (Nakanishi et al).

Moreover, heat-induced inflammation in pulp sensitizes pulpal afferent fibers by local release of prostaglandins; this effect is blocked by pretreatment with nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin,

**BOX 5-1 Peripheral mechanisms contributing to allodynia and hyperalgesia**

- Composition and concentration of inflammatory mediators
- Changes in afferent fiber: activation and sensitization
- Changes in afferent fiber: sprouting
- Changes in afferent fiber: proteins
- Tissue pressure
- Tissue temperature
- Sympathetic-primary afferent fiber interactions
- Aβ-fiber plasticity

Modified from Hargreaves et al 1995.

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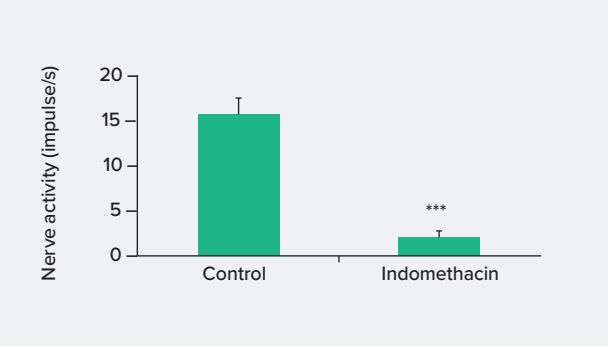


▲ **FIG 5-13** Effect of indomethacin (5 mg/kg) pretreatment on nerve activity after repeated administration of heat to the teeth of anesthetized cats. Cats were anesthetized, electrodes were placed in two dentinal cavity preparations, and heat was administered by a 1-second application of a stick of hot gutta-percha (about 90°C); the data were recorded after the third application of heat. ***$P < .001$ (*t* test). (Data from Ahlborg.)

naproxen, and diclofenac (Ahlborg) (Fig 5-13). Heat-induced inflammatory changes can result clinically from tooth preparation procedures performed with insufficient water spray, for example. From a clinical perspective, these studies support the use of the nonsteroidal class of analgesics to treat pulpitis (Hargreaves and Keiser).

Dental procedures such as the incision and drainage of an abscess or a pulpectomy may reduce pain by reducing concentrations of mediators as well as by lowering tissue pressure. Many inflammatory mediators found in inflamed pulp or periradicular tissue can either activate or sensitize nociceptors and evoke pain when administered to human volunteers. Considerable research is being directed toward clarifying the mechanisms of sensitization of nociceptors in the hope that this information will lead to the development of new classes of analgesic drugs (Eglen et al, Rang et al).

In addition to activation and sensitization, the peripheral afferent fibers respond to mediators such as nerve growth factor by increasing protein synthesis of substance P and CGRP and by sprouting terminal fibers in the inflamed tissue (Byers and Närhi, Byers et al, Byers 1994, Buck et al). Similar increases in neuropeptides are seen in human pulp infected by caries or following trauma and orthodontic forces (Boissonade, Hall et al, Chavarria-Bolanos et al). Sprouting of free nerve endings increases the density of innervation in inflamed tissue and may contribute to increased pain sensitivity in chronic pulpal or periradicular inflammation (Byers and Närhi, Byers et al). Elegant neuroanatomical studies have demonstrated that pulpal primary afferent fibers exhibit exuberant sprouting toward areas of insult such as dental caries (Couve et al) (Fig 5-14).

Certain afferent fibers also respond to inflammatory mediators by upregulating the expression of proteins such as voltage-gated sodium channels (Nav) that are important in nerve impulse formation and propagation (Wells et al, Warren et al). The different types of sodium channels can be classified based on sensitivity or resistance to tetrodotoxin (TTX) (Arbuckle and Docherty, Gold et al, Gold). An early study suggested a differential sensitivity of TTX-sensitive and TTX-resistant sodium channels to local anesthetics; the TTX-resistant subtypes (Nav1.8 and Nav1.9) were reportedly more resistant to lidocaine than TTX-sensitive forms such as Nav1.7 (Roy and Narahashi). More recent studies have shown that the Nav1.8 sodium channel subtype (a TTX-resistant form) is more sensitive to lidocaine than TTX-sensitive currents, including those associated with the Nav1.7 subtype (Leffler et al, Chevrier et al). Given these differences in the lidocaine sensitivity of sodium channel subtypes, the synthesis of new types of ion channels on sensory neurons may well contribute to the difficulty in obtaining local anesthesia that arises in certain cases of endodontic pain.

Although evidence exists for the involvement of all the major sodium channel subtypes that are preferentially expressed in the peripheral nervous system to inflammatory pain mechanisms, the Nav1.7 subtype appears to be especially important (Cummins et al). An inherited mutation of this gene results in the lack of a functional current and pain insensitivity in humans, while other point mutations have been identified in two different chronic pain syndromes (Cox et al, Goldberg et al, Yang et al, Fertleman et al). These findings suggest that Nav1.7 represents a promising target for the future development of a new class of analgesics.

Several other peripheral mechanisms may contribute to allodynia or hyperalgesia. For example, Aβ-fibers are normally thought to be low-threshold mechanoreceptors that do not convey nociceptive signals. However, under certain inflammatory conditions, Aβ-fibers begin to express substance P and develop new central terminations in the spinal cord dorsal horn, innervating regions that contain nociceptive neurons (Neumann et al, Coggeshall et al). Thus, it is possible that certain pain conditions may have an allodynic component because of nociceptive transmission by Aβ-fibers. Other studies have implicated sympathetic fibers in the activation of nociceptive afferent C-fibers after certain forms of tissue injury (Perl).

Taken together, several peripheral mechanisms contribute to the development of allodynia and hyperalgesia. However, not all these mechanisms are necessarily equally active in all acute and chronic pain states, and this factor has led to the concept of mechanistically based pain diagnoses (Hargreaves et al 1994, Woolf et al).


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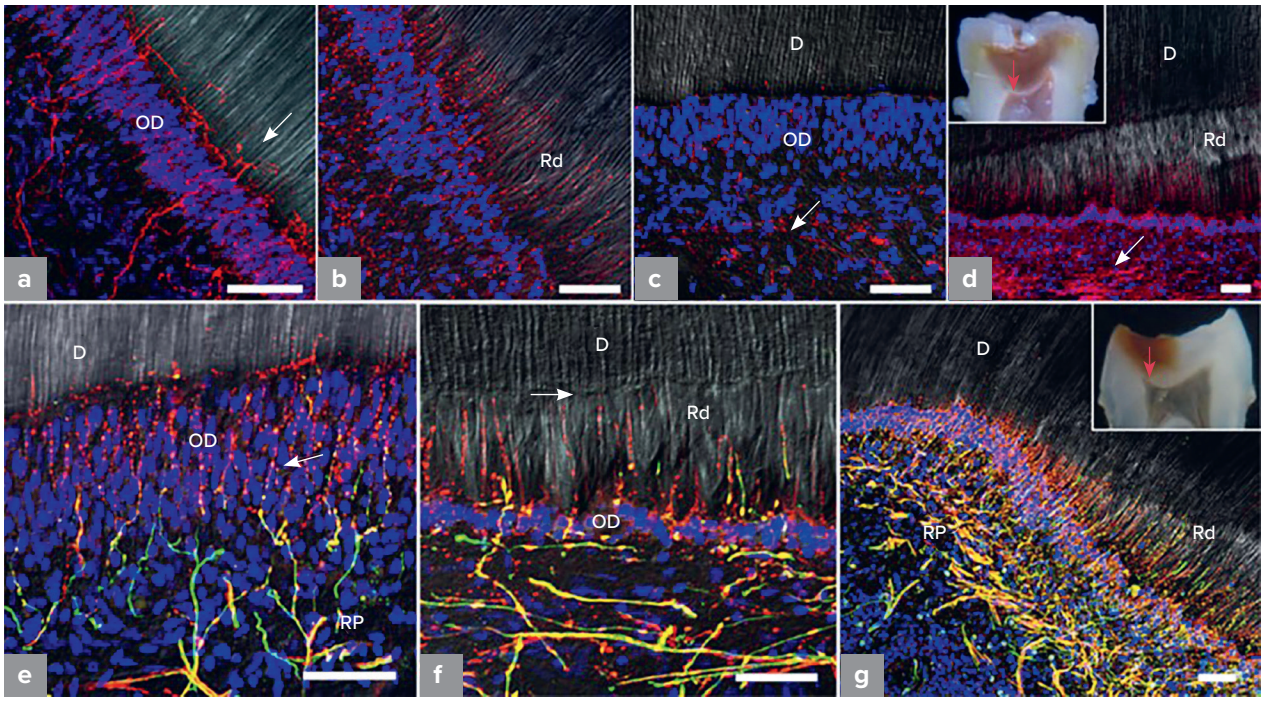


▲ **FIG 5-14** *(a)* Neuronal fibers (detected by βIII-tubulin staining in *red*) in a healthy section of the dentin-pulp complex show a well-developed branched subodontoblast nerve plexus extending axon profiles through the odontoblast layer (OD), forming a complex terminal network at the predentin-dentin interface *(arrow)*. *(b)* The innervation pattern changes in a carious tooth, with robust sprouting observed extending into the reactionary dentin matrix (Rd). *(c and d)* The expression of growth-associated protein 43 (GAP-43), associated with axonal growth, is sparse in the healthy pulp (*red* staining in *c*) but robust in sprouted fibers under carious lesions *(d)*. *(e)* Double immunolabeling of neurofilament *(green)* and βIII-tubulin *(red)* demonstrates that in the healthy dentin-pulp complex, nerve fibers forming the Raschkow plexus (RP) branch at the subodontoblast layer and form numerous varicosities between the odontoblasts *(arrow)*, extending vertically through the OD into dentin (D). *(f)* Nerve fibers sprout into reactionary dentin. *(g)* Robust sprouting of the Raschkow plexus is observed in a tooth with moderate caries. Scale bar = 50 µm.

### Central mechanisms of allodynia and hyperalgesia

In addition to these peripheral mechanisms, several central mechanisms of allodynia and hyperalgesia have also been proposed (Woolf and Costigan, Dubner and Basbaum, Woolf, Dubner and Ruda, Hargreaves et al 1995) (Box 5-2). These central mechanisms lead to a pain state called *central sensitizatio*n that is marked by the increased excitability of central neurons (Woolf). Central sensitization results from a barrage of impulses from C nociceptors. This results in the central release of glutamate and substance P (as well as other neurotransmitters), leading to activation of central receptors for glutamate (eg, NMDA and AMPA receptors) and substance P (eg, neurokinin 1 receptor) (see Fig 5-4). Under these conditions, stimulation of the normally low-threshold Aβ-fibers, normally involved in the transmission of non-noxious mechanical stimulation (eg, touch), produces a much larger response,

**BOX 5-2 Central mechanisms contributing to allodynia and hyperalgesia**

- Increased neurotransmitter release from primary afferent fibers
- Changes in postsynaptic receptors
- Changes in secondary messenger systems
- Changes in proto-oncogenes
- Changes in endogenous opioids or cannabinoids
- Central sensitization
- Reduced presynaptic inhibition
- Reduced postsynaptic inhibition
- Wind-up
- Dark neurons
- Activation of glia

Modified from Hargreaves et al 1995.

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resulting in pain, which may provide a central mechanism of allodynia.

Another important mechanism of central sensitization is related to the endogenous inhibitory tone that descending pathways exert on the synaptic transmission. These descending inhibitory neurons originate in the locus coeruleus, amygdala, and nucleus raphe magnus and have norepinephrine and 5-hydroxytryptamine as neurotransmitters. The inhibitory nature of these neurons underlies the analgesic efficacy of some tricyclic antidepressants or serotonin norepinephrine reuptake inhibitors in certain pain states. These drugs increase the concentrations of the inhibitory neurotransmitters (norepinephrine and 5-hydroxytryptamine) and thus the endogenous inhibitory tone in the trigeminal complex, resulting in the attenuation of the ascending excitatory nociceptive signals. On the other hand, suppression of the descending inhibitory pathways results in disinhibition of the central pain modulation and facilitation of ascending pain signals and greater pain perception (see Fig 5-4).

Several other mechanisms for central sensitization have been proposed (see Box 5-2). The concept of central sensitization is important because it implies a dynamic responsiveness of the central nervous system to peripheral input. Put another way, the same stimulus does not always produce the same response.

Activation of pulpal neurons produces a central sensitization (Sessle 2000, Chiang et al, Sunakawa et al). Application of mustard oil to the pulp of a rat maxillary molar activates pulpal neurons and evokes more than a fivefold increase in the discharge rate of nociceptive-specific neurons located in the nucleus caudalis (Chiang et al). This barrage of pulpal C-fibers is sufficient to produce a central sensitization because 20 minutes later there is an enhanced response to light tactile stimuli applied to the maxillary skin. This central sensitization is mediated by release of glutamate because pretreatment with an antagonist to the glutamate NMDA receptor (MK-801) abolishes this effect. Similarly, application of mustard oil to the rat molar dental pulp leads to the upregulation of NMDA receptors in the thalamus (Kawamura et al, Kaneko et al). Thus, activation of pulpal nociceptors can induce central sensitization via central increase of the expression of NMDA receptors and the release of glutamate in both the trigeminal nuclei and thalamus, and this effect is sufficient to produce allodynic-like responses (Chiang et al, Kaneko et al).

Pulpotomy can also evoke a central sensitization, as measured by both an expansion of receptive field sizes and an increase in spontaneous activity in the nucleus oralis (Kwan et al, Torneck et al) (Fig 5-15). In addition, central terminals of afferent fibers continue to exhibit increased release of CGRP, even after removal from the animal (Garry and Hargreaves). Thus, even in the absence of peripheral input, central mechanisms of hyperalgesia or allodynia can persist for some time. Importantly, central sensitization is a prominent clinical feature in patients; it has been identified in teeth contralateral to teeth with an endodontic diagnosis requiring intervention, particularly in teeth diagnosed with symptomatic irreversible pulpitis (Alelyani et al, Owatz et al).

Because at least some components of allodynia or hyperalgesia can persist even without continued sensory input from inflamed tissue, it is not surprising that up to 80% of patients experiencing pain before endodontic treatment continue to report pain after treatment (Marshall and Walton, Marshall and Liesinger). Thus, even if the endodontic treatment has removed all peripheral factors contributing to hyperalgesia, the central mechanisms can still persist for some time. Indeed, the presence of preoperative hyperalgesia (operationally measured as preoperative pain or allodynia) is a risk factor for the occurrence of postendodontic pain (Polycarpou et al, Torabinejad et al) (Fig 5-16). Patients with moderate or severe preoperative pain tended to report greater pain levels for 3 days after endodontic cleaning and shaping than did patients reporting either no or mild preoperative pain (Hargreaves and Hutter). A clinical study found that changes in the inhibitory descending pathways, resulting from altered central (spinal) modulation of nociceptive impulses, is likely involved in the symptoms of patients with long-term persistent pain following endodontic therapy (Nasri-Heir et al). This loss of inhibitory tone represents one of the numerous changes that could result in central sensitization. Because these plastic changes are more likely to take place in patients with moderate to severe preoperative pain, the presentation of this risk factor should alert the clinician to adjust the pain-management strategy for higher levels of pain control (Hargreaves and Hutter), which could include drugs that modulate central pain modulatory mechanisms such as pregabalin and gabapentin (Watkins et al 2009, Verma et al, Hill et al).

It has been reported that the overall incidence of pain 6 months or more after endodontic therapy is approximately 5.3%, of which 3.4% is of nonodontogenic origin (Nixdorf et al). Therefore, it is possible that some of these teeth were previously misdiagnosed and underwent unnecessary endodontic therapy or that the neuronal plasticity that resulted from a prolonged inflammatory state has not yet been reversed. Conversely, patients with persistent pain after endodontic therapy that has successfully removed the obvious disease etiology (ie, infection) may have pain states other than inflammatory pain, such as neuropathic pain, dysfunctional pain, or referred pain. The alarming number of patients with persistent pain


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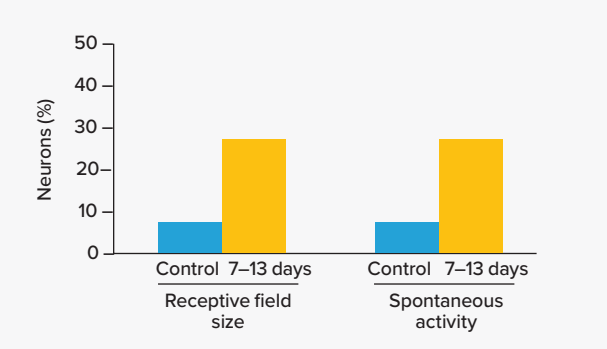
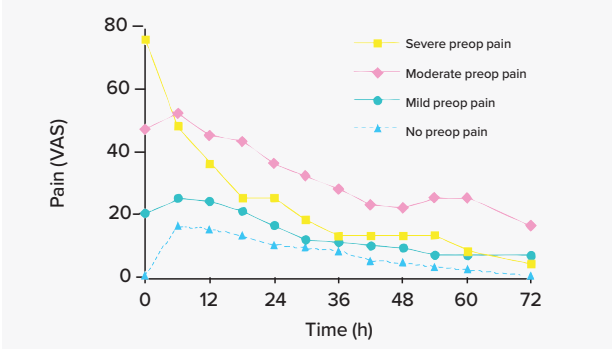



▲ **FIG 5-15** Effects of pulpotomy in a rat mandibular molar on a mechanoreceptive field and spontaneous activity of central neurons located in the trigeminal subnucleus oralis under control conditions and 7 to 13 days after pulpotomy. *$P < .05$ (chi-square test); **$P < .01$ (chi-square test). (Data from Kwan et al.)




▲ **FIG 5-16** Time-response curves for postendodontic pain in patients given a placebo analgesic. Patients were subdivided into four groups based on the magnitude of their preoperative pain. Patients with moderate or severe preoperative pain tended to report greater levels of pain throughout the postendodontic (cleaning and shaping) treatment. VAS = visual analog scale (0 to 100). (Data from Torabinejad et al.)

following an otherwise successful endodontic therapy highlights the need for an astute clinician to understand the pain pathways involved in both odontogenic pain (nociceptive and inflammatory pain states) and nonodontogenic pain (neuropathic, dysfunctional, and referred pain states) and to have a flexible pain-management plan to address these very different entities.

In summary, under normal physiologic conditions, nociceptors are only activated by suprathreshold noxious stimuli (nociceptive pain). The nociceptive pain signal acts as an important surveillance system, active when there is a stimulus of enough intensity to cause the individual harm. This signal remains active for as long as the stimulus is present. However, inflammatory pain may be manifested as hyperalgesia (increased responses to noxious stimuli) and mechanical allodynia (pain after non-noxious stimuli) (Fig 5-17). In the hyperalgesic state, the individual is made aware of a disease state by a greater-than-usual painful response. In the allodynic state, further stimulation of the area of inflammation, even low-intensity stimulation, results in pain, causing avoidance behavior and protection of the inflamed site (eg, patients often avoid chewing solid foods in the quadrant of a tooth with symptomatic apical periodontitis). Therefore, nociceptive and inflammatory pain protects from actual or potential damage and promotes healing. In addition, as discussed previously, both peripheral and central mechanisms govern these two pain manifestations. Lastly, central sensitization may underlie persistent painful states or a slower kinetic of pain resolution. Thus, astute clinicians can detect when central sensitization is suspected to better inform patients of expectations and provide a more individualized treatment plan.

## Neuropathic and dysfunctional pain

Pain states that neither protect nor promote healing may result from maladaptive plasticity of the peripheral and central nociceptive pathways. Both neuropathic and dysfunctional pain states are examples of this maladaptation of the pain pathways. Although there are similarities between these two pain states, they represent separate entities.

Neuropathic pain is typically caused by an identifiable initial presentation. The most prevalent type of orofacial neuropathic pain is trigeminal peripheral neuropathy. This painful condition could have a peripheral origination site, such as nerve damage following third molar extraction, administration of local anesthetics, implant placement, or orthognathic surgeries (Loescher et al, Tay and Zuniga), or, in the case of trigeminal neuralgia, a central origination site, such as central nerve compression, stroke, or a degenerative systemic disease (eg, multiple sclerosis) (Katusic et al, Kim, Baron et al). Although resolution of inflammatory pain is expected after the removal of the etiology (eg, infection), the same is not true for neuropathic pain. This pain state is the result of significant maladaptive neuronal

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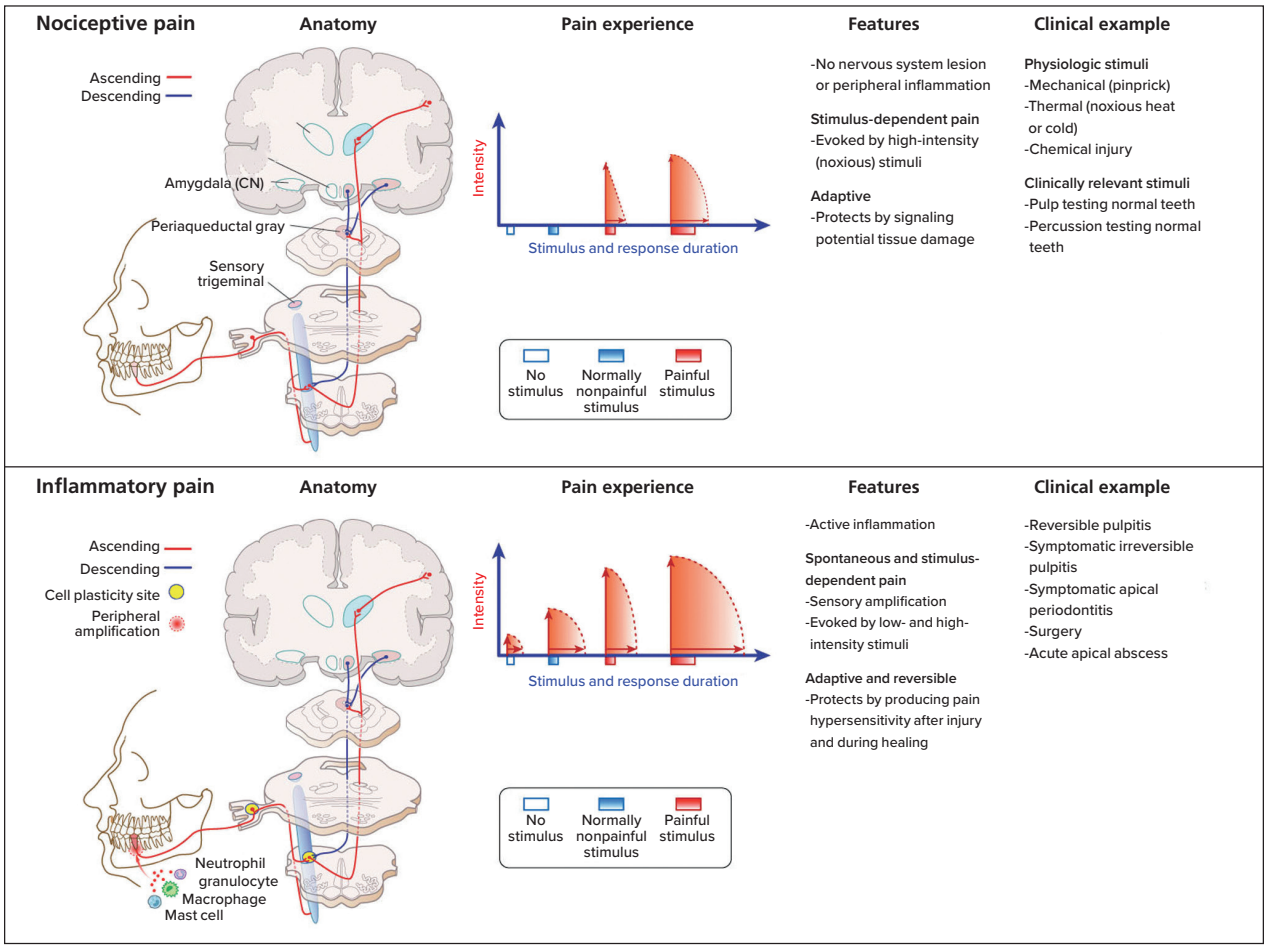


▲ **FIG 5-17** Mechanisms and characteristics of nociceptive pain and inflammatory pain. CN = central nucleus.

plasticity, and interventions are often palliative with the goal to improve the patient's quality of life.

Further peripheral nerve injuries appear to be directly related to the incidence of neuropathic pain (Richardson et al 1998b). In fact, the risk of developing neuropathic pain after nerve injury appears to be related to the size or diameter of the injured nerve. For example, nerve injury is common in endodontic therapy because pulpal and periradicular primary afferent neurons are routinely axotomized during root canal therapy and periradicular surgeries, respectively. In this case, a very small nerve bundle is severed rather than a larger neuronal bundle, such as the inferior alveolar nerve, which, as opposed to endodontic therapies, is significantly associated with the risk of developing neuropathic pain (Tay and Zuniga).

Although the incidence of persistent pain following adequate endodontic therapy is reported to be 5.6%, there has not been any investigation to determine whether these cases are due to a form of neuropathic pain. One important feature that aids in the diagnosis of these conditions is the quality of the perceived pain, often characterized as high-intensity "electric shock–like," "lancinating," or "stabbing" pain (Fig 5-18). These pain qualities noticeably differ from the usual mild-intensity "sharp" pain associated with nociceptive pain (eg, cold vitality test in an uninflamed tooth) or the "dull, aching" pain associated with inflammatory pain (eg, lingering response to cold vitality test in an inflamed tooth).

Another important feature of this painful presentation is its lack of response to anti-inflammatory drugs such as NSAIDs or acetaminophen. On the other hand, pregabalin and gabapentin, modulators of central nervous system activity, are relatively effective in the treatment of neuropathic pain states such as trigeminal neuralgia (Khan 1998,


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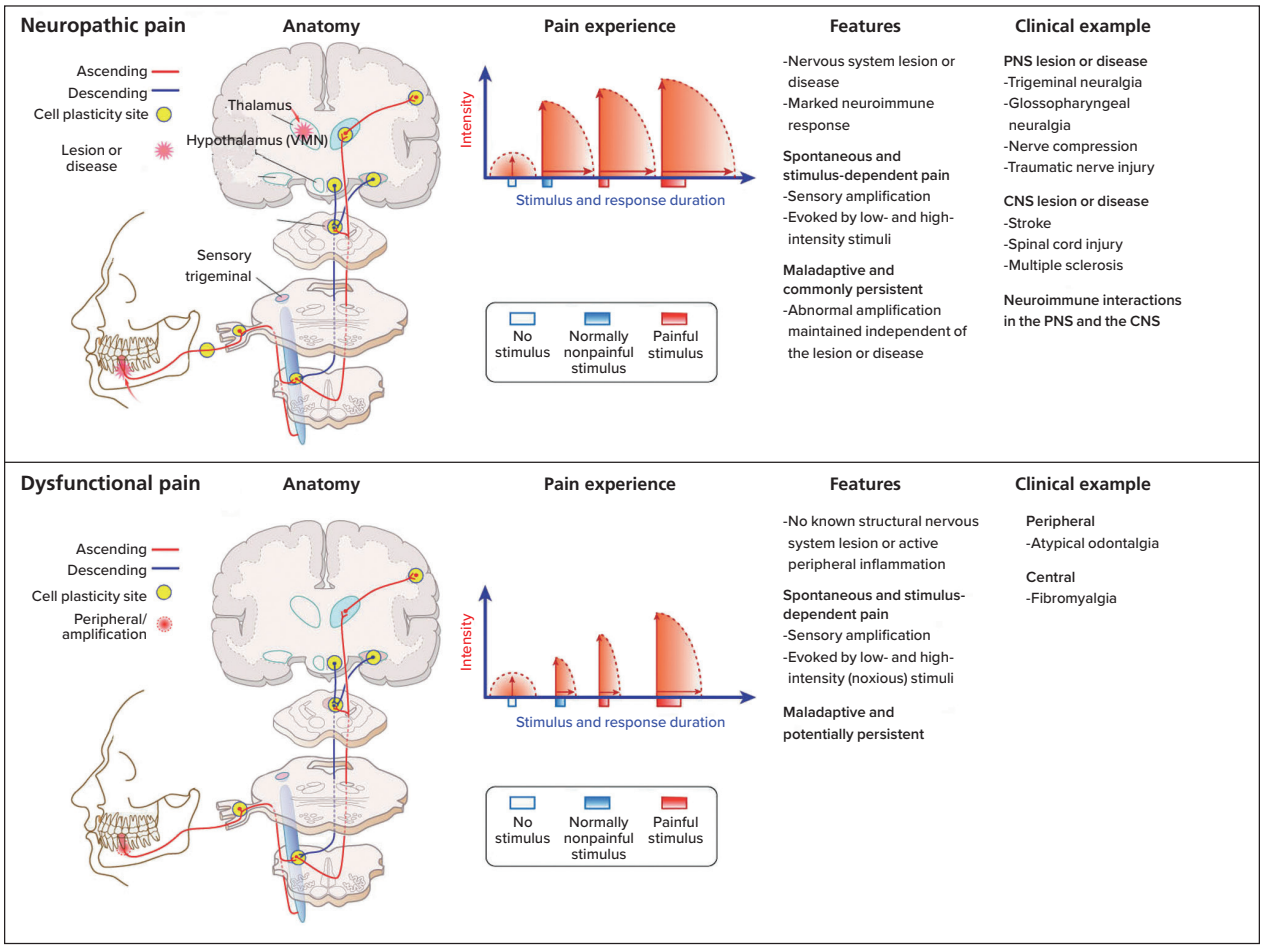


▲ **FIG 5-18** Mechanisms and characteristics of neuropathic pain and dysfunctional pain. VMN = ventromedial nucleus; CN = central nucleus; PNS = peripheral nervous system; CNS = central nervous system.

Obermann et al, Perez et al). The effect of these drugs in persistent endodontic pain following adequate endodontic therapy has not yet been evaluated. Therefore, clinicians and researchers should be alert to the mechanisms and properties of neuropathic pain states to adequately treat and research this painful and devastating condition.

Dysfunctional pain is another maladaptive chronic pain state, but it differs from neuropathic pain in not having a known cause. However, it shares many of the features of neuropathic pain, such as temporal summation with increased responses to repeated stimuli and lowered activation thresholds (see Fig 5-18). Thus, this largely unknown condition is characterized by peripheral amplification of low- and high-intensity stimuli. Examples of this presentation include fibromyalgia and may include atypical odontalgia (Graff-Radford and Solberg, Woda et al, Staud, Staud et al).

## Referred pain

Referred pain is the condition in which pain is perceived to be localized in one region but is caused by nociception originating from another area. In referred pain, the region of the body where pain is perceived to occur is not the same as the region where the pain originates. Clearly, referred pain represents a diagnostic challenge to the clinician because effective treatment must be directed to the site where pain originates.

Suda and colleagues have nicely summarized peripheral and central mechanisms of referred pain. Peripheral mechanisms include branching axons that innervate different structures and axonal reflexes (Suda et al). Central mechanisms include muscle contractions, central sensitization, memory, and convergence of primary afferents. These hypotheses are not mutually exclusive and may contribute

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to various cases of referred pain. For example, there is good experimental evidence that central sensitization and convergence of primary afferents may mediate many cases of referred pain (Khan et al 2007b, Sessle et al).

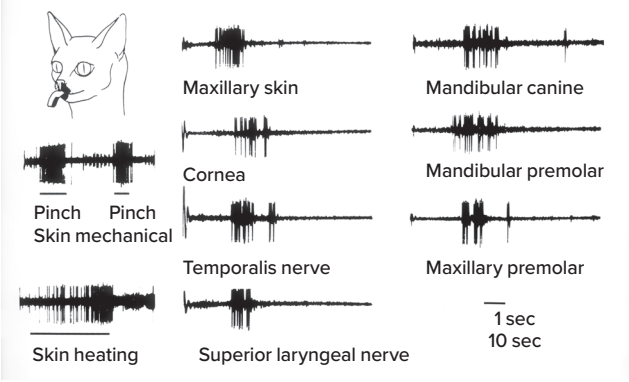
Evidence shows that the central terminals of trigeminal afferent fibers converge onto the same projection neurons. For example, nociceptors in the maxillary sinus and maxillary molars may stimulate the same neuron located in the nucleus caudalis (Sessle 2000, Sessle et al, Sessle 1986). About 50% of neurons in the nucleus caudalis exhibit convergence of sensory input from cutaneous and visceral structures (Sessle 1986). In one example, a single neuron in the nucleus caudalis received input from sensory neurons innervating the maxillary skin, cornea, a mandibular canine, a mandibular premolar, and a maxillary premolar (Sessle et al) (Fig 5-19). Thus, the clinical problem of referred pain has a biologic basis in the convergence of sensory neurons onto the same central projection neuron, making it difficult for the patient to discern where the pain originates. Indeed, it has been reported that more than 26% of patients experiencing odontogenic pain are unable to accurately localize the painful tooth (McCarthy et al).

The theory of convergence has been used to explain the clinical observation of a patient who complains of pain that originates from an inflamed mandibular molar and radiates to the preauricular region or of pain that originates in inflamed maxillary sinuses and radiates to the maxillary posterior teeth. Thus, pain can originate from the dental pulp and be referred to distant regions or, conversely, can originate from distant regions and be referred to the dental pulp.

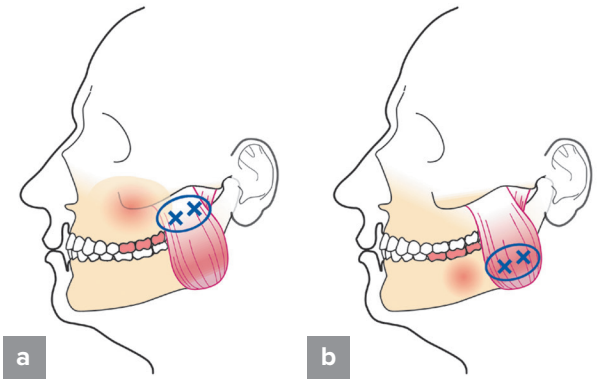
The latter has been demonstrated in patients with chronic orofacial pain. In patients with trigger points in the superior belly of the masseter muscle, pain can be referred to maxillary posterior teeth (Travell and Simons) (Fig 5-20). Conversely, in patients with trigger points in the inferior border of the masseter muscle, pain can be referred to the mandibular posterior teeth. Thus, it is important to consider multiple diagnostic tests to determine the origin of a patient's pain.

In addition to convergence, another important mechanism of referred pain is central sensitization. Nociceptive signals that originate in the periphery but have increased intensity and duration may lead to changes in the central terminals, such as upregulation of NMDA receptors for the excitatory neurotransmitter glutamate and release of substance P and other mediators, as already discussed. These changes may lower the threshold of activation of projection neurons that also receive inputs from nociceptors that innervate uninflamed regions (convergence). This decreased threshold can potentially lead to pain when uninflamed teeth experience innocuous stimuli (eg, mastication) and low-intensity signals are amplified by a sensitized central projection neuron. Clinically, this phenomenon can be observed when a whole quadrant is tender to percussion while only a single tooth is inflamed and is the culprit for this presentation. Interestingly, mechanical allodynia has been detected using a sensitive quantitative method in otherwise normal teeth contralateral to the inflamed tooth (Khan et al 2007b).

Both convergence and central sensitization are likely to take place concomitantly and form the basis for the diagnostic use of local anesthetics in establishing the origin of




▲ **FIG 5-19** Example of convergence of multiple sensory neurons onto the same central neuron in the nucleus caudalis of an anesthetized cat. The receptive field for the neuron was in the maxillary region. The figure shows the depolarizations of the neuron in the nucleus caudalis to stimulation in various orofacial regions. (Reprinted with permission from Sessle et al.)




▲ **FIG 5-20** Referral patterns in patients with chronic orofacial pain. *(a)* Patients with trigger zones in the superior belly of the masseter muscle have pain referred to regions including the maxillary posterior teeth. *(b)* Patients with trigger zones in the inferior belly of the masseter muscle have pain referred to regions including the mandibular posterior teeth. (Redrawn after Travell and Simons with permission.)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pain in patients who present with diagnostic challenges. For example, Okeson described the selective injection of local anesthetics as a clinical test to distinguish the site of pain origin from the area of pain referral (Okeson).

## Mechanisms of Dentinal Hypersensitivity

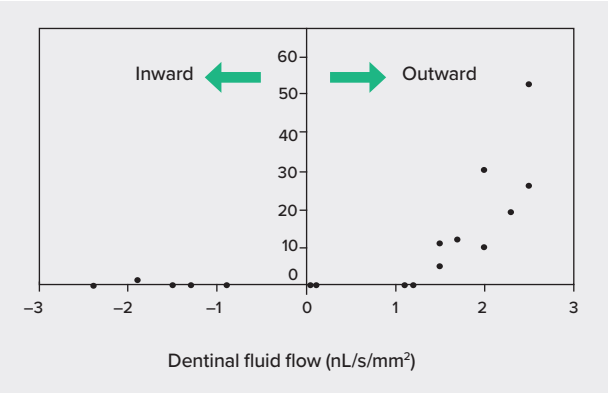
### Hydrodynamic theory of dentinal hypersensitivity

Dentinal sensitivity is characterized as a sharp pain that occurs soon after a provoking stimulus (Ahlquist and Franzén 1994a). Dentinal tubules are well innervated near the pulp horns. In this region, up to 74% of the tubules contain nerve fibers, and these fibers can extend up to approximately 200 μm into the tubule (see Fig 5-2). At the midcrown level of the pulp, fewer tubules are innervated, and the intratubule extension of the fibers is shorter. In contrast, root dentin is poorly innervated.

For all innervated tubules, the nerve fibers are in close proximity to odontoblasts, although direct connections are not evident (Byers and Närhi). However, the proximity of these two cell types is consistent with the hypothesis that odontoblasts and afferent terminal endings may have biochemical connections (eg, via expression of receptors and paracrine release of soluble factors) and thereby participate in the sensory transduction of noxious stimuli.

This anatomical relationship between nerve fibers and odontoblasts may be the physiologic basis for the hydrodynamic theory of dentinal pain. This theory, which has strong experimental support, postulates that movement of fluid through the dentinal tubules results in pain (Brännström and Aström, Brännström et al, Trowbridge, Orchardson and Gillam). Stimuli including air blasts, cold, and hypertonic sugars ("sweets") can produce movement of the dentinal fluid. Movement of this fluid results in stimulation of nociceptive nerve fibers located on the pulpal side of the dentinal tubules (Matthews and Vongsavan, Matthews et al) (Fig 5-21). The fluid movement is thought to serve as a fluid transducer, signaling the presence of stimuli at the outer opening of the dentinal tubules. Indeed, removal of dentinal fluid abolishes the ability to detect these stimuli; sensitivity returns when the fluid is replenished (Lilja et al).

It is not known how nerve fibers detect fluid movement. However, the sharp quality of the resulting pain suggests activation of Aδ nociceptive fibers (Närhi 1985, Närhi et al 1992a, Ahlquist and Franzén 1994b) (see Figs 5-7 and 5-8). Interestingly, more recently it has become clear that dentinal tubules are also innervated by low-threshold Aβ-fibers that normally convey responses to light touch and proprioception (Fried et al, Emrick et al). These fast-conducting, heavily myelinated fibers are likely involved in the detection of subtle changes in fluid movement within the dentinal tubules, while the Aδ-fibers are more likely to respond to high-threshold stimulation. It is important to mention that until recently the molecular mechanism by which mechanical force was detected by sensory neurons was largely unknown. This changed with the discovery of the mechanogated ionotropic channel Piezo2 that is expressed preferentially in mechanosensitive primary afferent fibers (Ranade et al). This channel has been found to be expressed in sensory neurons innervating the dental pulp and dentinal tubules (Emrick et al, Han et al). Thus, mechanotransduction of fluid movement likely involves the activation of Piezo2, allowing cationic influx, neuronal depolarization, and the generation of action potentials that are rapidly conducted by lightly and heavily myelinated fibers (Aδ- and Aβ-fibers).




▲ **FIG 5-21** Effect of velocity of dentinal fluid flow on the responses of an Aδ-fiber that innervates dentin in the anesthetized cat. (Data from Matthews et al.)

Dentinal sensitivity appears to recede with age or after chronic irritation. The increase in secondary or reparative dentin during these processes is thought to diminish the flow of fluid through the tubules (Dowell et al). This anatomical change explains the difficulty in eliciting a response upon cold testing a tooth with radiographic signs of secondary or tertiary dentinogenesis.

Following exposure of dentin, either by loss of enamel or by loss of cementum and gingiva, dentinal sensitivity can develop. Researchers have reported that there is a large increase in the responsiveness of Aδ-fibers to dentinal stimulation after an acid-etching procedure is performed on exposed dentin (Närhi et al 1992b) (Fig 5-22). The responsiveness to mechanical (eg, probing), drying (eg, air blast), and osmotic stimuli is increased. The buccal surfaces of canines and premolars are common sites of dentinal exposure, probably because of their susceptibility to toothbrush-induced abrasion (Orchardson and Collins).

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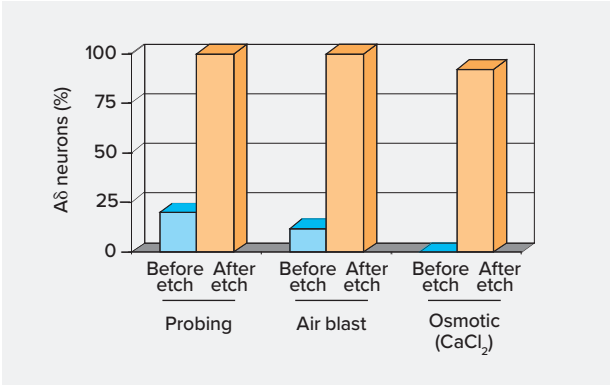
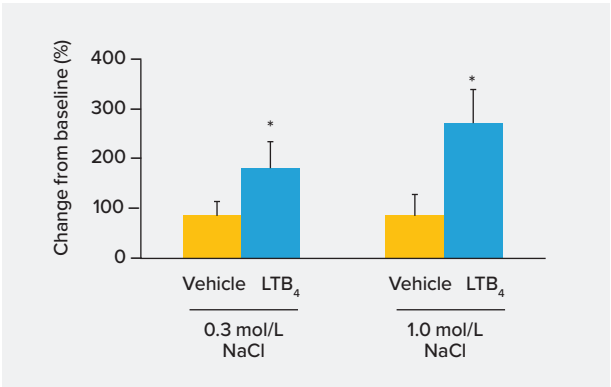
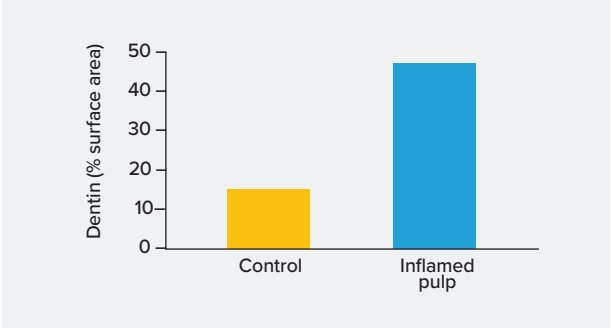



▲ **FIG 5-22** Effect of acid etching on the responsiveness of Aδ afferent fibers to stimuli applied to exposed dentin in anesthetized dogs (N = 23 to 25). $CaCl_2$ = calcium chloride. (Based on Närhi et al 1992a, Närhi et al 1992b.)




▲ **FIG 5-23** Effect of administration of leukotriene $B_4$ ($LTB_4$) or vehicle on dentinal sensitivity to osmotic stimuli. $LTB_4$ (25 mg/mL) or vehicle was applied to exposed dentin in anesthetized cats; electrodes were placed in cavities for intradentinal recordings. Administration of $LTB_4$ significantly enhanced responsiveness to osmotic stimuli (0.3 and 1.0 mol/L of sodium chloride [NaCl]) applied to the exposed dentin. *$P < .05$ (ANOVA) versus vehicle. (Data from Madison et al.)




▲ **FIG 5-24** Effect of pulpal inflammation on the receptive field size of Aδ-fibers innervating the dentin of dog teeth. The teeth underwent resection of the coronal portion of the crown 1 week before the experiment. On the day of the experiment, dogs were anesthetized, single units were isolated, and the exposed dentin was probed to determine the area of innervated dentin. (Data from Närhi et al 1996.)

## Effects of pulpal inflammation on dentinal sensitivity

Dentinal sensitivity is not an invariant sensation. The responsiveness to dentinal stimulation increases not only with exposure of dentinal tubules (see Fig 5-22) but also in the presence of inflammatory mediators. For example, the administration of the inflammatory mediator leukotriene $B_4$ to deep dentinal preparations increases the responsiveness to osmotic stimuli (Madison et al) (Fig 5-23). In an actual model of pulpal inflammation, there is a threefold increase in the receptive field size of Aδ-fibers innervating inflamed dog teeth (Närhi 1985, Byers and Närhi, Närhi et al 1996, Närhi et al 1994) (Fig 5-24). This may result from the sprouting of Aδ-fibers and the resultant increase in innervated dentinal tubules. Thus, pulpal inflammation may predispose a tooth to enhanced dentinal sensitivity by reducing the threshold for activation and by increasing the area of innervated dentinal tubules.

# Pulpal Vitality Testing

One aspect of the diagnosis of pain is the determination of pulpal vitality. The importance of assessing vascular integrity to establish pulpal vitality is discussed elsewhere in this book. However, methods for assessing vascular integrity are generally not well validated or amenable to all clinical applications. Accordingly, responsiveness to various pulpal stimuli has been used as a surrogate measure of pulpal vitality (Rowe and Pitt Ford). In general, this involves the use of electrical or thermal testing or preparation of a test cavity; the patient's response is an outcome measure.

Accepted techniques, such as the use of control teeth interspersed with the suspected tooth, have been described in clinical texts, and the reader is encouraged to review this material (Cohen and Liewehr). This section reviews factors that modify outcomes in electrical and thermal testing and reviews studies on sensitivity and specificity.

Although most commercially available electrical pulp testers are monopolar (ie, cathode on tooth and anode often on the lip), studies have shown that bipolar electrodes (ie, cathode and anode placed on tooth) provide more consistent effects (Virtanen et al, Virtanen, Mumford and Bowsher 1976). For example, the coefficient of variation (a measure of the noise-signal ratio) for bipolar electrode stimulation of Aδ-fibers is about 35% less than that for monopolar stimulation of the same units (Virtanen).

The density of the electrical current also plays an important role in this test. A larger surface area of electrodes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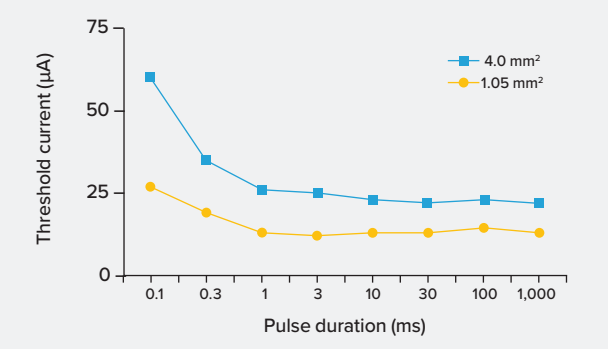
requires greater current to produce a detectable sensation, and, at any given electrode area, shorter pulse widths require greater current to produce a sensation (Mumford and Newton) (Fig 5-25). This is an important concept because the density of the current is the result of these parameters as well as the pulp anatomy.

When results in different types of teeth or results from younger patients and older patients (with smaller pulp chambers) are compared, the current delivered cannot be directly compared because the pulp anatomy differs. Studies have indicated that electrical testing produces the most consistent effects when the electrode is placed on the incisal or cuspal edge of the tooth (Jacobson and Arbor, Bender et al). Other factors that influence electrical testing include the restorative status of the tooth (eg, porcelain is an insulator) and even the patient's health (hypertensive patients have significantly higher thresholds for electrical testing) (Ghione et al).

Alternative testing methods include the application of cold or hot stimuli to the tooth (Ahlquist and Franzén 1994a, Ahlquist and Franzén 1994b, Ikeda et al, Ikeda and Suda, Alquist et al 1984, Ahlquist and Franzén 1999). Commonly used cold stimuli include ethyl chloride spray, dichlorodifluoromethane, dry ice (ie, frozen carbon dioxide), and wet ice (ie, frozen water). In general, the response to application of cold stimuli is measured as a positive or negative reaction. This technique is probably used more often than application of hot stimuli. Commonly used hot stimuli include electrical heat sources and heated gutta-percha (applied to a lubricated tooth surface). The initial response to heat is a sharp sensation; if the stimulus is maintained for a sufficient period of time, a dull, aching sensation is perceived. These sensations appear to be mediated by Aδ- and C-fibers, respectively.

All clinical tests are subject to false-positive (ie, a positive response from a necrotic pulp) and false-negative (ie, a negative response from a vital pulp) results (Hyman and Cohen, Petersson et al). One study compared the cold test (ethyl chloride) and an electrical pulp test to the "gold standard" (endodontic access and clinical verification of vitality) in 59 teeth of unknown pulpal status (Petersson et al). The probability that a negative test meant a true necrotic pulp was similar for the cold and electrical tests (89% versus 88%); the hot gutta-percha test exhibited a much lower ability to detect a true negative (48%). The probability that a positive test represented a true vital pulp was similar for the cold, electrical, and hot gutta-percha tests (90%, 84%, and 83%, respectively). Overall, the cold and electrical tests had similar accuracy values (86% versus 81%), and both were more accurate than the heat test (71%).

Other studies have reported that the cold test is more accurate (80%) in testing human teeth (N = 50) than the electrical test (64% for monopolar electrodes) (Moody et al). In one study of more than 1,000 teeth, two different electrical testers each produced significantly more (about fivefold higher) false-positive results than did the cold test. Even when gingival controls were employed, one commercial electrical tester produced about twofold greater false-positive results than the cold test (Peters et al).




▲ **FIG 5-25** Effect of monopolar electrode and stimulation parameters on the threshold for pain perception in humans. Electrodes (1.05-mm² or 4.0-mm² surface area) were placed on exposed dentin, and the parameters were evaluated to determine if they caused positive test responses (ie, pain perception threshold of electrical stimulus). (Data from Mumford and Newton.)

## Conclusion

The mechanisms of pulpodentinal pain have numerous clinical implications. First, dentinal pain is primarily caused when the myelinated fibers innervating dentinal tubules detect fluid movement and signal that movement back to the brain. Therapeutic reduction of dentinal fluid movement or neuronal activation can reduce dentinal hypersensitivity. Second, inflammation is detected by receptors expressed on pulpal nociceptors; the binding of inflammatory mediators onto these receptors can activate or sensitize these nociceptors. Drugs that reduce tissue levels of inflammatory mediators (eg, NSAIDs) relieve pain by reducing activation of these receptors. Third, hyperalgesia and allodynia can occur during pulpal and periradicular inflammation. Evaluation of the presence of these altered pain states provides the biologic basis for endodontic diagnostic tests. Fourth, hyperalgesia and allodynia can occur by both peripheral and central mechanisms and may persist beyond the dental appointment. Thus, patients with preoperative pain have an increased risk of experiencing postoperative pain. Fifth, maladaptive pain states such as neuropathic pain (eg, trigeminal neuralgia) and dysfunctional pain (eg, atypical odontalgia) are devastating conditions that result from significant plasticity of the peripheral and central nervous system; they represent a diagnostic and therapeutic challenge.

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Sixth, referred pain is due in part to convergence of multiple sensory fibers onto the same central projection neuron and may be associated with central sensitization. Seventh, pulp testing, using either electrical or thermal stimuli, requires an understanding of the mechanisms involved to allow proper interpretation of tooth and patient conditions and to minimize confusion caused by false-positive or false-negative results.

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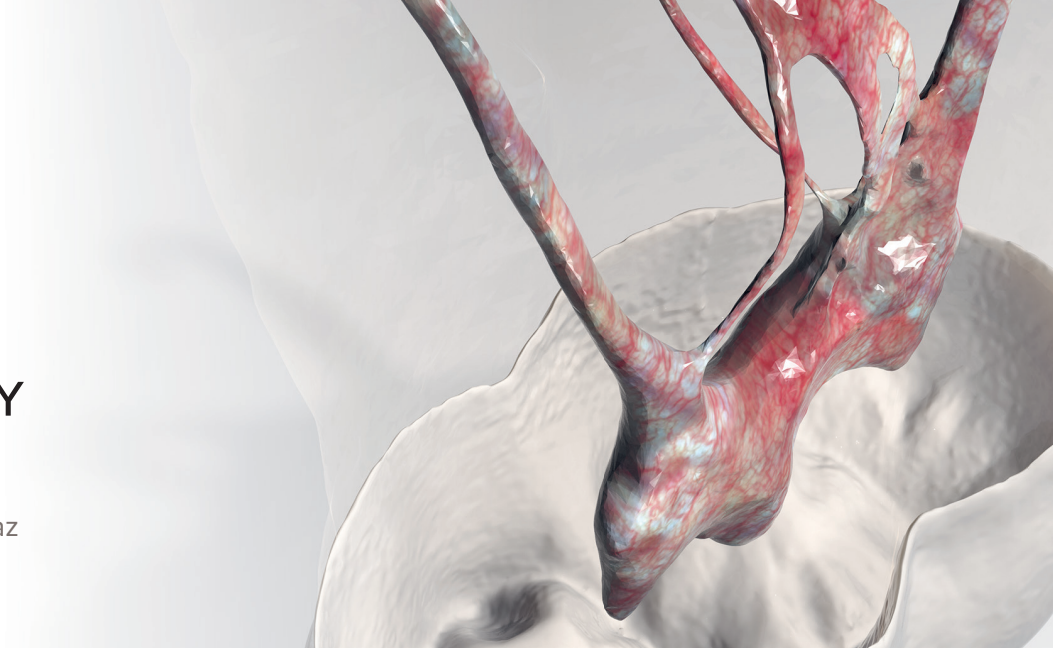
# 6

# ENDODONTIC MICROBIOLOGY

Brenda P.F.A. Gomes
Maria Teresa Arias-Moliz
Luis Eduardo Chavez de Paz
Ronald Ordinola Zapata



The dental pulp consists of a well-vascularized and innervated connective tissue, encased in rigid walls, that is in communication with the periodontal ligament and alveolar bone through the apical foramen. Like many connective tissues, the dental pulp presents defensive mechanisms that allow it to react to bacterial contamination, mainly from caries. If pulpal irritants are not removed, the pulpal response could progress from a transient inflammation (reversible pulpitis) to irreversible pulpitis and pulp necrosis (Nair 1987, Gomes, Gomes and Herrera). Clinically, pulpal damage may occur without the presence of symptoms (Michaelson and Holland). Without root canal intervention, bacteria can populate the total extension of the root canal system (Nair 1987).

The periapical tissues react to this bacterial insult with an inflammatory response known as *apical periodontitis*, which results in bone resorption. Although not desirable, bone resorption also has a protective function in the context of endodontic infections. This idea is supported by the observations that *(1)* bacteria are not found in large numbers in the periapical tissues and *(2)* osteomyelitis is a rare complication of apical pathosis. Bacteria that can enter the periapical tissues are exposed to antibodies, the complement system, and the cells of the immune system. This results in bacterial lysis and phagocytosis by polymorphonuclear cells and/or macrophages (Nair 2004, Gomes and Herrera). In some scenarios, an acute apical abscess may develop and spread to neighboring facial areas. The local damage depends mostly on the virulence of the causative bacteria and the immunocompetence of the host. This type of oral infection has the potential to cause severe life-threatening complications (Flynn et al, Gomes and Herrera).

Although several bacterial species have been identified in endodontic infections, their composition and diversity are distinct from bacterial communities found in other biologic niches, such as saliva or caries (Costalonga and Herzberg). Numerous factors that are present in the root canal influence the taxonomic composition of endodontic infections, including the nutrients present (such as proteins and inflammatory exudate), pH, temperature, and oxidation-reduction (redox) potential (Sundqvist and Figdor). Firmicutes, Actinobacteria, Fusobacteria, Proteobacteria, Spirochaetes, and Bacteroidetes are the most abundant phyla detected in root canal infections (Siqueira et al 2011, Manoil et al). In this chapter, we will discuss the role that microbial communities play in caries and endodontic infections.

## General Characteristics of Oral Biofilms

The term *microbial communities* has been traditionally used to refer to the group of microorganisms living together. Specifically, microbial communities are defined as collections of multiple species, where microorganisms interact with each other in a connected environment (Konopka). The term *microbiota* describes the microorganisms thriving in a specific ecosystem, including bacteria, fungi, archaea, protists, and algae (Marchesi and Ravel). On the other hand, the term *microbiome* was first introduced by Whipps et al in 1988. In 2020, new definitions for *microbiome* and *microbiota* were proposed (Berg et al). The term *microbiome* now refers to a distinct microbial community that occupies a well-defined habitat with specific physical and chemical properties. The microbiome not only includes the microorganisms themselves but also encompasses their activities, leading to the formation of unique ecologic niches. The microbiome is a dynamic and interactive microecosystem that can change

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over time and scale. It is integrated into larger macroecosystems, such as eukaryotic hosts, where it plays a crucial role in their functioning and health (Berg et al). The *microbiota* comprises microorganisms from various kingdoms, including prokaryotes (bacteria, archaea) and eukaryotes (plants, animals, fungi, algae, protozoa, and other complex organisms). The "theater of activity" of the microbiota encompasses microbial structures, metabolites, mobile genetic elements (such as transposons, phages, and viruses), and residual DNA that are influenced by the environmental conditions of the habitat (Berg et al). Figure 6-1 shows a schematic highlighting the composition of the *microbiome*.

Bacteria inhabit the oral environment in two different states: *(1)* in a free-floating (planktonic) mode and *(2)* adhered to surfaces, forming biofilms (dental plaque) (Kolenbrander et al 2010). Biofilms are commonly described as a community of microorganisms that are embedded in a self-produced extracellular matrix (Costerton et al). The planktonic mode is only transient as bacteria are transported mainly by saliva to colonize different surfaces of the oral cavity. Bacteria in planktonic mode are more vulnerable to stressors and must swiftly modify their phenotype to establish an adherent mode in order to survive (Kolenbrander et al 2010). The phenotype of planktonic bacteria in the oral cavity is now widely accepted to be significantly different from the phenotype of the same organism attached to surfaces (Jakubovics and Kolenbrander). When bacteria detect the presence of a surface to be colonized, various phenotypic changes occur in the cell. These phenotypic alterations are controlled by processes that allow bacteria to attach to surfaces and form colonies. Some of these cellular mechanisms include the expression of cell membrane receptors or the enhancement of binding structures such as flagella (Chang). Once oral bacteria are firmly adhered to the substrate, secondary colonization and formation of microcolonies takes place (Kolenbrander et al 2002, Kolenbrander et al 2010). In biofilms, oral bacteria thrive in heterogenous communities providing them with a wide variety of possibilities to adapt and survive, resulting in complex structures of oral bacteria embedded in a thick layer of self-produced matrix (Beikler and Flemmig).

## Bacterial Identification

To enhance the understanding of oral biofilm communities, it is essential to identify and characterize the microbiota causing endodontic pathosis. Bacteria are single-celled microorganisms, typically measuring around 0.2 µm in diameter and 2 to 8 µm in length. They present different shapes, ranging from spheres to rods and spirals. Traditionally, they have been observed using microscopic

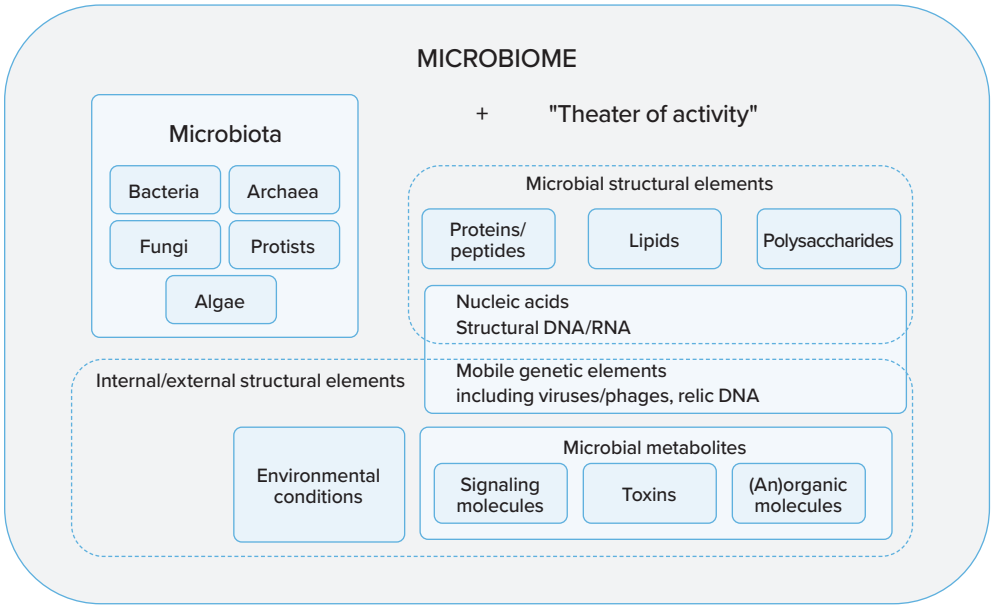


**FIG 6-1** Diagram highlighting the composition of the microbiome, containing both the microbiota (community of microorganisms) and their "theater of activity" (structural elements, metabolites/signaling molecules, and the surrounding environmental conditions) (Berg et al).


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techniques, including light, electron, transmission, dark-field, atomic force, and expansion microscopy (Yoshizawa et al, Rumyantseva et al).

Initially, the identification of microorganisms in endodontic specimens mostly relied on culture techniques. This method comprises the isolation, cultivation, and biochemical characterization of microorganisms. However, this approach may underestimate the incidence of certain nonculturable bacteria (Nóbrega et al 2016a). In addition, the cultivation of a microorganism does not necessarily ensure its correct identification. Culture-based identification is dependent on studying the phenotypic characteristics of reference strains, which exhibit predictable biochemical and physical traits under ideal growth conditions (Siqueira et al 2007, Nóbrega et al 2016a). However, various phenotype-related characteristics can offer obstacles to identification, sometimes limiting it to the genus level.

The development of techniques based on polymerase chain reaction (PCR) and its derivatives, as well as DNA-DNA hybridization, led to substantial advances in molecular diagnostic procedures during the 1990s. One of the primary advantages of these methods is their capability to detect nonculturable microorganisms. Culture sensitivity typically ranges from approximately $10^4$ to $10^5$ microbial cells in a nonselective medium and $10^3$ cells in a selective medium. PCR sensitivity, on the other hand, ranges from 10 to $10^2$ cells. Nested PCR can identify a species even when 10 cells are present in the sample (Siqueira and Rôças 2005a). Open molecular approaches that can detect all bacteria present in a sample, including previously unknown species, offer the most robust methods for exploring the microbial profile in a microbial community (Kumar et al). Until a few years ago, conventional molecular techniques were only able to identify a restricted component of the microbiota, specifically the cultivable portion, which accounted for approximately 5% of the microbial community (closed-end molecular methods). The advent of open-end molecular methods improved microbial analysis by enabling access to the global microbiota through the analysis of DNA extracted from mixed samples. The recent introduction of nucleic acid sequencing methodologies has opened the ability to generate millions of DNA sequences in a single sequencing run. This technology, known as *next-generation sequencing* (NGS), is rapidly transforming the microbiology field by providing immediate answers to a wide range of analyses. NGS enables parallel sequencing of multiple microbiologic samples through PCR amplification of a phylogenetic marker, such as the 16S rRNA gene (Caporaso et al). It also enables faster and more accurate detection of both abundant and rare members of the microbial community (Caporaso et al). Major open-end molecular methods include 16S rRNA sequencing, shotgun metagenomics, and RNA sequencing.

Direct amplification and sequencing of the 16S rRNA regions have led to the deposition of over 700 orally derived 16S sequences in GenBank, with less than half of them corresponding to cultured and characterized species (Kumar et al). The 16S rRNA amplicon sequencing technique is typically based on the amplification of small fragments covering one or two hypervariable regions (such as V1-V3, V3-V4, V4, or V4-V5 regions) of the 16S rRNA genes of bacteria/archaea. The DNA sequences of these amplicons are then mapped to a reference 16S sequence database using bioinformatics for taxonomic identification and relative abundance estimation (Zhang et al 2021). Compared to amplicon sequencing, whole-metagenome shotgun sequencing analyzes *all* the DNA in a given sample. This includes other domains such as fungi, parasites, and viruses. The readings obtained through these methods are compared against primary reference databases (RefSeq) that contain all publicly available genomes (O'Leary et al). Compared to shotgun sequencing, 16S rRNA NGS techniques tend to offer lower resolution and sensitivity for detecting species-level changes and cannot detect changes at the strain level (Durazzi et al, Shah et al, Poretsky et al). While NGS techniques can provide genus-level taxonomic assignments, whole genome sequencing (WGS) remains the gold standard for high-resolution microbiome analysis and functional characterization of the human microbiome (Knight et al 2018, Ordinola-Zapata et al 2024).

## Dental Caries

Dental caries is a ubiquitous disease caused by a dysbiotic oral microbiota that creates an acidic environment adjacent to the tooth surface, leading to the demineralization of hard tooth structure (Pitts et al). It is a multifactorial biofilm-mediated chronic disease whereby poor nutritional habits, poor oral hygiene, and the presence of caries-related microorganisms in combination with inadequate fluoride exposure result in the formation of the cariogenic ecosystem (Chen et al 2021). Caries is considered the most common human disease, affecting 80% to 90% of the world population (Petersen). According to global estimates, 621 million children had untreated cavities in the dentin of primary teeth and 2.4 billion people had untreated cavities in the dentin of permanent teeth in 2015 (Kassebaum et al). Evidence shows that there is an association between low socioeconomic status and a higher risk of having dental caries (Schwendicke et al).

For decades, *Streptococcus mutans* was considered the main causative agent of the disease (Loesche et al 1975).

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As a consequence, most diagnostic, preventive, and therapeutic strategies have been targeted toward this microorganism. However, as will be explained later in the chapter, the introduction of NGS methods to study the microbiome associated with caries revealed that this species accounts for a small portion of the cariogenic bacterial community. Caries occurs as an imbalance between the host and the complex and highly diverse saccharolytic carious microbiome (Simón-Soro and Mira).

## Pathogenesis

Dental caries starts with demineralization of the enamel by the action of organic acids produced by the bacteria in the dental biofilm. These acids are obtained from the metabolism of dietary fermentable carbohydrates, lactic acid being the most important one involved in caries formation (Pitts et al). As the pH drops in the biofilm, the acids diffuse into the enamel crystals, and the tooth surface starts demineralizing. This demineralization can be reversed by calcium, phosphate, and fluoride deposition, which promotes a new mineral crystal surface (remineralization) that is much more resistant to acid than was the original hydroxyapatite mineral (Pitts et al).

Dental caries is a dynamic disease process that involves continuous demineralization and remineralization cycles throughout the day (Featherstone). These demineralization and remineralization processes are influenced by several different factors, including sugar intake, composition of saliva, and fluoride concentration. The cariogenic effect of sugar usually depends on how effectively sugar can be metabolized by the bacteria in the dental biofilm. Thus, monosaccharides such as glucose and fructose directly enter the glycolytic pathway, inducing a rapid decrease in pH, whereas other complex carbohydrates such as starch are first converted into simpler carbohydrates prior to entering the glycolytic pathway (Takahashi and Nyvad 2011). Consequently, simple sugars tend to be more cariogenic than complex ones (Gasmi et al). Saliva is an innate defense system of the human body that protects teeth from caries development through mechanisms such as remineralization, neutralization of low pH, antibacterial properties, and rinsing capacity of sugar accumulation (Gasmi et al). As an example, saliva contains urea and peptide, which can be metabolized by oral bacteria to generate ammonia, thereby increasing the pH (Kleinberg). Saliva is also rich in histatins and phosphate, which buffer the biofilm pH and protect against caries development.

## Ecological plaque hypothesis

Different hypotheses have been proposed to explain the relationship between dental plaque and the development of oral diseases. One of the most accepted theories about the etiology of dental caries is the extended ecological plaque hypothesis (Takahashi and Nyvad 2008, Takahashi and Nyvad 2011). This hypothesis states that supragingival biofilm consists mainly of non-mutans streptococci and *Actinomyces* spp that tend to maintain a neutral pH by the production of acidic and alkaline metabolites that neutralize the acid, resulting in the balance between demineralization and remineralization (the dynamic stability stage). However, the regular exposure of dental biofilm to fermentable dietary sugars results in repeated conditions of low pH, upsetting the balance toward demineralization. At this stage (the acidogenic stage), more acidogenic bacteria proliferate, such as low-pH streptococci and *Actinomyces* spp. These bacteria show acid tolerance thanks to a series of adaptive responses, including an increase in the proton pump activity of the cell membrane, the activation of the ATPase, the induction of metabolic pathways involved in alkalization, and the activation of stress proteins that protect enzymes and nucleic acids from acids. If this acidic environment is prolonged, more aciduric bacteria such as mutans streptococci and non-mutans aciduric bacteria (like lactobacilli and bifidobacteria) become dominant (the aciduric stage), which will induce the progress of the caries lesion (Takahashi and Nyvad 2016).

In root and dentin caries, bacterial acidification also induces the exposure of the organic matrix and the activation of dentin-embedded and salivary proteases such as metalloproteases and cathepsins (Takahashi and Nyvad 2016). These proteases may initiate a partial degradation of the acid-exposed tooth organic materials, composed mainly of collagen, which can be further broken down by the bacterial proteolytic activity and by the acids. Thus, root and dentin caries harbor both acidogenic and aciduric bacteria, such as mutans streptococci, *Lactobacillus* and *Bifidobacterium* spp, as well as proteolytic bacteria, such as *Prevotella*, *Propionibacterium*, and *Fusobacterium* spp (Aas et al, Takahashi and Nyvad 2016). Dental caries, therefore, is not an example of a classic infectious disease but is a consequence of an ecologic shift in the balance of the resident oral microbiota promoted by changes in the oral environment (Pitts et al).

## Cariogenic bacteria

Cariogenic microorganisms usually show the ability to *(1)* rapidly transport sugars when in competition with other plaque bacteria, *(2)* convert sugars to acids, and *(3)* maintain these activities even under low-pH conditions (Marsh et al). They also produce intracellular and extracellular polysaccharides that can be further used as a nutrient source when nutrient availability is scarce. Extracellular polysaccharides are mostly involved in the


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adhesion of the bacteria to the tooth surface and aggregation with other microorganisms.

Traditionally, cariogenic bacteria included species of the genus *Streptococcus*. They are considered initial colonizers of dental plaque and quantitatively one of the most important microorganism groups within the oral ecosystem. *Streptococcus mutans* is a common etiologic agent of caries due to a relevant sugar metabolism that includes a competitive ability to intake and ferment sugar (Chen et al 2021) and the production of soluble and insoluble glucan extracellular polymers (Hajishengallis et al). The level of *S mutans* in the saliva is positively correlated with the degree of dental caries (Belstrøm et al), although caries may also occur in the absence of *S mutans* (Baker et al). Other *Streptococcus* species, such as *Streptococcus sobrinus,* have also been considered cariogenic due to their acidogenic and aciduric properties (Gross et al).

Lactobacilli are acidogenic and aciduric bacteria that are involved more in the progression of the caries lesion than the initiation (Torlakovic et al, Belstrøm et al, Rôças et al 2016). Lactobacilli are not high producers of extracellular polysaccharides. Consequently, lactobacilli show low adherence to smooth tooth surfaces, and their high occurrence in retentive areas may be due mainly to mechanical retention. *Bifidobacterium* spp and *Scardovia wiggsiae* are also considered etiologic agents in the cariogenic supragingival biofilm based on their capability for sugar fermentation and aciduric condition (Torlakovic et al 2012, Belstrøm et al, Chen et al 2021).

Several mechanisms are involved in the acid tolerance of *S mutans* and other aciduric species. Some of these mechanisms include the activity of the ATPase, which maintains intracellular pH at 7.5; the action of the urease and arginine deiminase, which produces ammonia to increase the pH; and its ability to convert L-malate to L-lactate plus $CO_2$ and adenosine triphosphate (ATP), which contributes to deacidifying the cytosol and provides energy for $H^+$ translocation into the extracellular environment (Peterson et al).

## Bacterial diversity in caries

For decades, the sugar-fermenting aciduric bacteria described previously have been considered the main etiologic factors of dental caries. However, recent high-throughput sequencing analyses of caries lesions have revealed an extraordinarily diverse ecosystem where *S mutans*, *Lactobacillus* spp, and other aciduric bacteria account for only a small fraction of the bacterial community (Simón-Soro and Mira). This supports the concept that the ecosystem is composed of multiple microorganisms that act collectively in the infection. The 16S rRNA and metagenomics sequencing have shown that the microbiome associated with caries lesions has significantly less bacterial diversity compared to the microbiome associated with health (Baker et al). In addition to *S mutans* and *Lactobacillus* spp, species of *Prevotella*, *Veillonella*, *Atopobium*, *Schlegelella*, and *Pseudoramibacter* (Belda-Ferre et al, Simón-Soro and Mira, Belstrøm et al) seem to be more prevalent in patients with caries (Peterson et al, Baker et al), while genera such as *Rothia*, *Neisseria*, *Haemophilus*, *Leptotrichia*, *Fusobacterium*, and *Porphyromonas* are more prevalent in caries-free patients (Xu et al, Baker et al). Interestingly, *Veillonella* spp are not considered an etiologic agent of dental caries. However, the association observed between cariogenic bacteria and *Veillonella* spp stems from their metabolic requirement for organic acids, such as lactic acid, which are found in higher concentrations in active caries (Zhou et al).

Apart from bacteria, fungi like *Candida* spp are also associated with caries (Ramadugu et al, Xiao et al). The presence of sugars stimulates the interaction between *S mutans* and *Candida albicans*, resulting in the development of an extracellular matrix that provides binding sites for *S mutans* and enables *C albicans* to colonize the surface of the tooth (Koo et al). Viruses like human herpesvirus 4 (Epstein-Barr virus) also showed some correlation with caries, although their role is yet to be studied (Baker et al).

A recent systematic review (Bhaumik et al) showed that when comparing the caries- and health-related microbiomes according to age, *Streptococcus sanguinis*, *Fusobacterium periodonticum*, *Granulicatella elegans*, *Gemella haemolysans*, and *Haemophilus parainfluenzae* were significantly associated with oral health in children, whereas no specific species related to health condition was observed in adults. *S mutans*, on the other hand, was significantly related to cariogenesis in adults and children, supporting the importance of this species in these lesions.

In order to have a better understanding of caries, it is important to know how the microbiome functions. A reduced diversity of microbial functional pathways related to health was observed in the caries-associated microbiomes, including the depletion of arginine biosynthesis (Nascimento et al), branched-chain amino acids (Santiago et al), urea (Liu et al), and lipid metabolisms (Belstrøm et al, Baker et al). On the other hand, glycan biosynthesis and carbohydrate metabolisms were upregulated (Belstrøm et al). Caries-free samples or healthy sites instead showed an upregulation of metabolic routes involved in the neutralization of acids such as dehydrogenase and methylenetetrahydrofolate reductase genes (Duran-Pinedo). Caries-related microbiomes can also be modulated by ecologic factors such as sugar intake and frequency (Moynihan and Kelly, Tian et al 2015).

Enamel caries lesions harbor a lower diversity than "open" dentin cavities that were exposed to the oral

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cavity as well as "hidden" dentin cavities (Simón-Soro et al 2014). Enamel caries bacteria have an overexpression of dietary sugar-fermenting genes and adhesion molecules, whereas dentin caries organisms are enriched in genes involved in the glycans metabolism and proteases to degrade dentin tissue, including collagenases, dipeptidyl peptidases, metallopeptidases, and serine proteases (Simón-Soro et al 2013). In a recent study, Cho et al conducted an extensive analysis of supragingival biofilm collected from 416 preschool-age children. The goal was to identify and verify interactions between species inside the biofilm that are related to caries. Sixteen different taxonomic groups were shown to be linked to dental caries in children. The authors focused specifically on the dynamics of biofilm formation, spatial arrangement, and metabolic activity of *Selenomonas sputigena*, *Prevotella salivae*, and *Leptotrichia wadei* individually and in combination with *S mutans*. Their findings revealed that *S sputigena*, an anaerobic bacterium with flagella, plays an important role in the supragingival biofilm by becoming trapped in streptococcal exoglucans. Although it loses its motility, *S sputigena* actively proliferates within the biofilm, forming a honeycomb-like multicellular structure that encapsulates *S mutans* (Cho et al). This interaction increases acid production, which contributes to the acidogenic environment associated with caries formation. While *S sputigena* is incapable of generating caries on its own, when co-infected with *S mutans*, it significantly increases the severity of enamel lesions and accelerates the course of caries in vivo.

Overall, the microbiome associated with dental caries shows diverse bacterial compositions, interactions, and functions that vary dramatically in the same individual and between individuals, even within the same caries lesion type (Simón-Soro and Mira). This means that caries has a diverse etiology, there is no single pathogen responsible for the development of the lesion, and the microorganisms of the consortia act collectively (Simón-Soro and Mira). Scientists and clinicians tend to think in binary terms when forming cause-and-effect relationships. However, the problem of dental caries is likely to be significantly more complex, and many species in specific combinations will dictate the signatures associated with dental health and disease or the transition between the two states (Peterson et al).

## Prevention

Prevention of dental caries can be achieved by acting on the different etiologic factors that are involved in its development. Effective strategies include changing oral hygiene habits to disrupt the biofilm; applying fluorides; reducing sugar intake or using alternative sweeteners such as xylitol, which cannot be metabolized to acid by oral bacteria; applying sealants; and promoting periodic comprehensive oral health (Jepsen et al). There is evidence that combining different strategies to control the biofilm increases the efficacy. For example, the combination of improved oral hygiene with the use of fluorides is more effective in reducing the incidence of dental caries than improved oral hygiene alone (Sälzer et al, Hujoel et al). However, the risk of fluorosis in children and the consequent decreased acceptance of fluoride has led to the development of different alternatives. Hydroxyapatite- and nanohydroxyapatite-containing toothpastes are fluoride-free alternatives that have been shown to be effective as anticaries agents, although their efficacy on caries prevention has been questioned (Wierichs et al).

The fact that the caries-associated microbiome varies at different stages of disease progression and between individuals (Rupf et al) and that different taxonomic compositions give rise to similar functional profiles suggests that the same functions may be performed by different groups of microorganisms (Simón-Soro and Mira). This means that preventive and treatment strategies should be focused on modulating the microbial biofilm and/or its functional output rather than on the microorganisms involved. Some strategies include the disruption of the exopolymeric matrix and the inhibition of the quorum sensing, intercellular communication signals, carbohydrate-fermenting pathways, adhesion compounds, and pH-regulating systems (Simón-Soro and Mira). Disrupting exopolysaccharide (EPS) production may help to disperse the dental biofilm. Several mechanisms have been proposed for this, including enzyme degradation, eDNA and proteins, nanoparticles, and phage therapy (Lin et al 2022). However, their application is still limited due to the paucity of clinical trials or in vivo studies.

Additionally, the use of probiotics and prebiotics can modify microbial interactions or buffer cariogenic effects, favoring a healthy and eubiotic ecosystem (Simón-Soro and Mira). Thus, arginine has been shown to prevent dental caries by buffering the acids produced by cariogenic bacteria (Agnello et al, Nascimento). This amino acid is metabolized by arginolytic species present in the biofilm, producing ATP and ammonia and thereby raising the pH of the biofilm (Agnello et al). Inorganic $NO_3$ is also emerging as an effective prebiotic that alters the oral microbiota by increasing the abundance of oral health–associated $NO_3$, reducing genera *Neisseria* and *Rothia* and decreasing caries-associated bacteria such as *Streptococcus* spp (Bahadoran et al). Inorganic $NO_3$ supplementation also increases the salivary pH and prevents carbohydrate-induced acidity.


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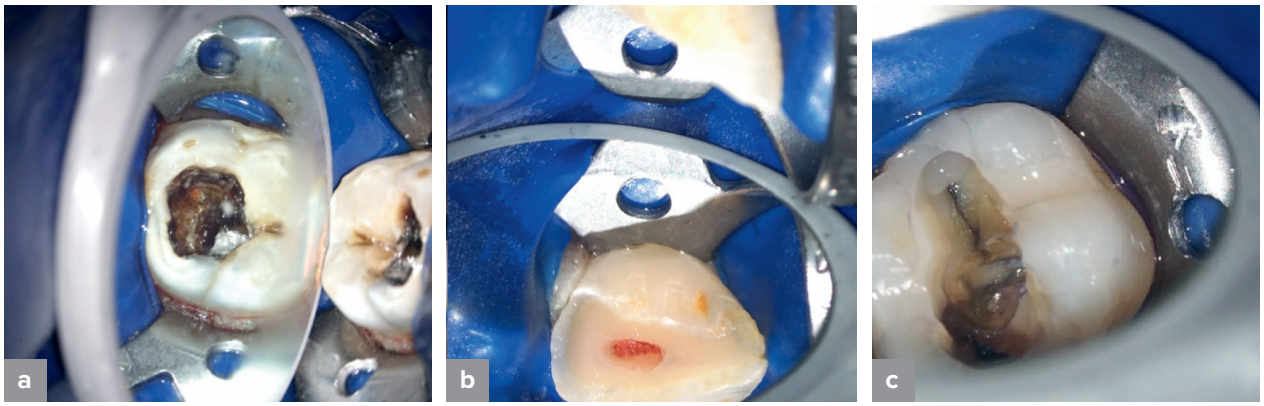

▲ **FIG 6-2** *(a to c)* Bacterial contamination of the pulp canal space can be the result of caries exposure, traumatic dental injuries, or cracks and fractures.

## Endodontic Infections

Oral bacteria have proved to be in the causal pathway of pulpal disease and apical periodontitis (Kakehashi et al). Root canal bacteria and their byproducts present antigenic properties that induce immunologic reactions in the periapical tissues, resulting in bone resorption. The microbial communities associated with endodontic infections have a dynamic characteristic and can be modulated by local factors (ie, previous endodontic treatment). Endodontic infections can be classified into primary and secondary/persistent endodontic infections. According to the location, they can be intraradicular or extraradicular. Extraradicular infections include acute apical abscesses and extraradicular biofilms that are originated by a primary or secondary/persistent intraradicular infection (Siqueira and Rôças 2022, Gomes and Herrera, Manoil et al).

### Primary endodontic infections

Primary endodontic infections are caused by bacteria that initially colonize and invade the necrotic pulp canal space. The composition and diversity of the microbiota in these infections are relatively consistent, regardless of the infection's origin, such as dental caries or trauma (Fig 6-2). Although bacterial rates may vary initially, over time the microbiota within root canals tends to favor the dominance of gram-negative and gram-positive strict anaerobes (Brook et al, Sundqvist 1976, Sundqvist 1992a, Sundqvist 1992b, Sundqvist 1994, Van Winkelhoff et al 1985, Van Winkelhoff et al 1992, Gomes, Gomes et al 2015, Gomes and Herrera, Siqueira and Rôças 2022). The microbial diversity in the root canals is different from other sites in the oral cavity and is driven by the specific ecologic conditions of the necrotic root canal space. Local factors favor the growth of anaerobic and proteolytic species. Although the overall community composition of the root canal microbiome is constant, there is high interindividual variability in terms of species composition and relative abundance (Siqueira and Rôças 2022). Similar to the oral cavity, over 500 different bacterial phylotypes have already been detected in infected root canals by culture or by molecular methods. In addition, about 40% of the bacteria found in infected canals have yet to be cultivated and fully characterized (Siqueira and Rôças 2022).

Bacteria in infected root canals belong to 20 phyla according to current studies (Gomes et al 2022). Strict anaerobic gram-positive species, including *Eubacterium*, *Eggerthella*, *Filifactor*, *Parvimonas*, *Peptostreptococcus*, *Pseudoramibacter*, and *Propionibacterium (Arachnia)*, are present. Additionally, facultative anaerobic gram-positive species are also found, including *Actinomyces*, *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Gemella*, and *Staphylococcus*. Among the gram-negative bacteria, some are strict anaerobes, including *Fusobacterium*, *Porphyromonas*, *Prevotella*, *Olsenella*, *Tannerella*, and *Treponema*. Others are facultative anaerobes, such as *Capnocytophaga*, *Eikenella*, *Haemophilus*, and *Neisseria* (Gomes and Herrera). Table 6-1 shows the bacterial genera in endodontic infections based on Gram staining, morphology, and gaseous requirements. Box 6-1 shows the microorganisms most commonly isolated/detected from root canals distributed according to phyla.

### Formation of biofilms in root canals

Root canal biofilms have been identified and described to form on root canal walls, dentinal tubules, apical deltas, and even the radicular cementum (Nair 1997, Chavez de Paz 2007, Ricucci and Siqueira 2010). The formation of biofilms on root canal surfaces has been hypothesized to follow a similar process as oral biofilms—that is, bacterial

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**TABLE 6-1 Bacterial genera commonly occurring in endodontic infections according to Gram-staining characteristics and gaseous requirements**

| Bacterial morphology | Obligate anaerobe(s) | Facultative anaerobe(s) |
|---|---|---|
| Gram-positive cocci | *Parvimonas, Peptoniphilus, Peptostreptococcus* | *Enterococcus, Gemella, Staphylococcus, Streptococcus** |
| Gram-negative cocci | *Veillonella* | *Neisseria* |
| Gram-positive rods | *Actinomyces, Eggerthella, Eubacterium, Filifactor, Lactobacillus, Olsenella, Propionibacterium, Pseudoramibacter* | *Actinomyces*, Corynebacterium, Lactobacillus*, Propionibacterium** |
| Gram-negative rods | *Alloprevotella, Bacteroides, Campylobacter, Dialister, Fretibacterium, Fusobacterium, Porphyromonas, Prevotella, Tannerella, Treponema* | *Capnocytophaga, Eikenella, Haemophilus* |

*Some species can also be strict anaerobes.

**BOX 6-1 Microorganisms most commonly isolated/detected from root canals distributed according to phyla**

**Firmicutes**
*Dialister pneumosintes*
*Dialister invisus*
*Eggerthella lenta*
*Enterococcus faecalis*
*Filifactor alocis*
*Finegoldia magna*
*Parvimonas micra*
*Peptoniphilus asaccharolyticus*
*Peptostreptococcus anaerobius*
*Pseudoramibacter alactolyticus*
*Selenomonas sputigena*
*Streptococcus anginosus*
*Streptococcus constellatus*
*Streptococcus intermedius*
*Streptococcus mitis*
*Streptococcus sanguinis*
*Veillonella parvula*

**Actinobacteria**
*Actinomyces gerencseriae*
*Actinomyces israelii*
*Actinomyces naeslundii*
*Actinomyces odontolyticus*
*Actinomyces viscosus*
*Propionibacterium acnes*
*Propionibacterium propionicum (Arachnia)*
*Slackia exigua*

**Fusobacteria**
*Fusobacterium necrophorum*
*Fusobacterium nucleatum* subsp *nucleatum*
*Fusobacterium nucleatum* subsp *polymorphum*

**Proteobacteria**
*Campylobacter gracilis*
*Campylobacter rectus*
*Eikenella corrodens*
*Haemophilus aphrophilus*

**Spirochaetes**
*Treponema amylovorum*
*Treponema denticola*
*Treponema lecithinolyticum*
*Treponema maltophilum*
*Treponema medium*
*Treponema pectinovorum*
*Treponema socranskii*
*Treponema vincentii*

**Bacteroidetes**
*Alloprevotella tannerae*
*Porphyromonas endodontalis*
*Porphyromonas gingivalis*
*Prevotella denticola*
*Prevotella intermedia*
*Prevotella melaninogenica*
*Prevotella nigrescens*
*Tannerella forsythia*

**Synergistetes**
*Fretibacterium fastidiosum*
*Pyramidobacter piscolens*

cells in a planktonic mode will sense the surface and attach themselves to organically coated surfaces before forming microcolonies (Nair 1997, Svensäter and Bergenholtz, Chavez de Paz 2007, Ricucci and Siqueira 2010) (Figs 6-3 to 6-5). Root canal biofilm formation begins after oral microorganisms invade the pulp chamber, using the pulp tissue and inflammatory breakdown products as a fluid vehicle to colonize deeper areas of the root canals (Svensäter and Bergenholtz). Light microscopy studies have confirmed that oral bacterial invaders are embedded in the pulpal inflammatory masses in primary endodontic infections (Ricucci and Siqueira 2010). It is suspected that inflammatory exudates play a crucial role in root canal biofilm formation because they serve as a liquid vehicle and as a substrate coating that promotes bacterial adherence to dentin and cementum surfaces. Ex vivo studies of extracted teeth with attached periapical lesions have confirmed the presence of root canal biofilms (Nair 1987)


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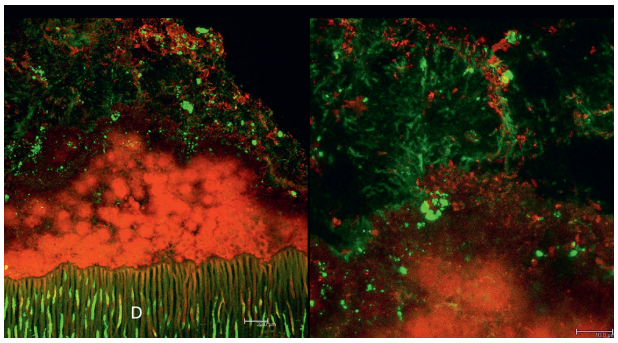
using various identification techniques such as histopathology (Ricucci and Siqueira 2010), scanning electron microscopy (Chavez de Paz 2007), and transmission electron microscopy (Nair et al 1999). All these investigations have accumulated evidence of the production of dense clusters of bacterial cells encased in extracellular matrix along the root canal walls of the dentin. Examples of the histopathology and taxonomy of primary endodontic infections can be found in Figs 6-6 to 6-8.

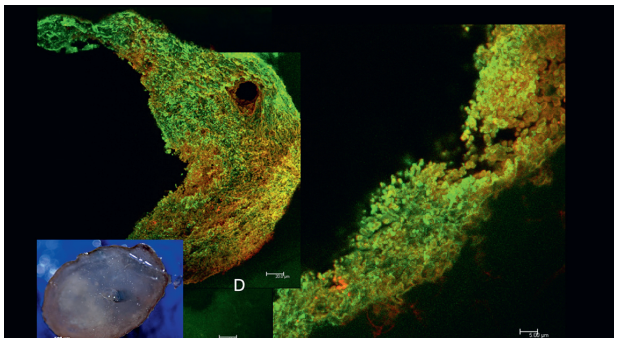
Certain bacteria can have commensal or antagonistic connections, depending on whether the growth of one organism promotes the other bacteria (positive association) or whether the relationship inhibits the growth of both species (negative association). Black-pigmented anaerobes (*Prevotella* and *Porphyromonas* spp) have particularly specific nutritional requirements: vitamin K and hemin. *Campylobacter rectus* has been demonstrated to enhance the growth of *Porphyromonas* spp by generating a growth factor related to hemin (Sundqvist 1994). A favorable association between *Porphyromonas*/*Prevotella* spp and *Campylobacter* spp in root canals has also been observed in infected root canals (Sundqvist 1994). Microbial associations between species can be determined by computing the odds ratio of detecting one species in the presence or absence of another species in culture medium. Root canal samples subjected to such analysis show very strong positive associations between certain bacterial species. *Fusobacterium nucleatum*, which is a highly prevalent species in endodontic infections, was positively associated with *Peptostreptococcus micros* (today *Parvimonas micra*), *Porphyromonas endodontalis*, *C rectus*, and *S sputigena* (Sunqvist 1992a, Sundqvist 1992b). These associations are most likely based on the nutritional demands of the bacteria.

Coaggregation, which is the adhesion of taxonomically distinct microbial taxa to one another in a synergistic path of biofilm formation (Horiuchi et al), has also been previously reported between two of the most abundant taxa found in endodontic infections: *Parvimonas* and *Fusobacterium*. *F nucleatum* expresses various surface adhesins, allowing it to bind to multiple species of oral bacteria and host cells (Kaplan et al 2009, Kaplan et al 2010, Kaplan et al 2014, Lima et al). These adhesins would not only allow *Fusobacterium* to persist in the root canal after treatment but could also contribute to the recruitment and retention of other oral bacteria (Kolenbrander et al 2006). *F nucleatum* is a potent stimulator of proinflammatory cytokines (Han et al, Park et al) and coaggregates with *P micra* (Horiuchi et al) and other microorganisms, potentially contributing to biofilm colonization of the root canal space.

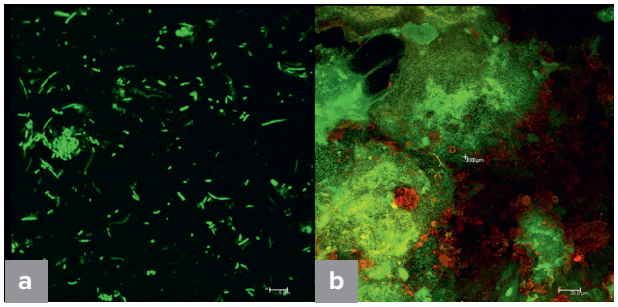
Ricucci and Siqueira (2010) concluded that apical periodontitis fulfills the criteria commonly used to establish




▲ **FIG 6-3** Confocal laser scanning microscopy (CLSM) photomicrograph revealing a biofilm architecture found in a single-rooted tooth diagnosed with pulp necrosis. The biofilm is firmly attached to the dentin structure. Observe how the biofilm presents multiple layers. Close to the substrate, microbial cells showing viability are surrounded by membrane-damaged cells labeled in *red*. Several bacterial morphotypes can be observed several micrometers away from the root canal wall. These cells are not permeable to the propidium iodide dye and present intact membranes. Live/dead staining technique; bars represent 10 µm. (Courtesy of Marco A.H. Duarte and Marcia S. Graeff; reprinted with permission from Ordinola-Zapata et al 2022.)




▲ **FIG 6-4** CLSM photomicrograph showing the microbial colonization of the apical third in a single-rooted tooth. The biofilm structure is composed of multiple bacterial morphotypes that are irreversibly attached to the surface of the root canal walls.




▲ **FIG 6-5** Oral bacteria in the planktonic and biofilm stage. *(a)* Antimicrobials can diffuse freely in a liquid environment and affect floating bacteria. *(b)* Biofilms are tridimensional structures that are irreversibly attached to a substrate (dentin or cementum). Antimicrobials do not diffuse well in this organic structure, and the biofilm matrix will react to the antimicrobial by reducing its concentration (ie, chlorine consumption). Microbial cells in deep biofilm layers may not be affected as much by the antimicrobial challenge.

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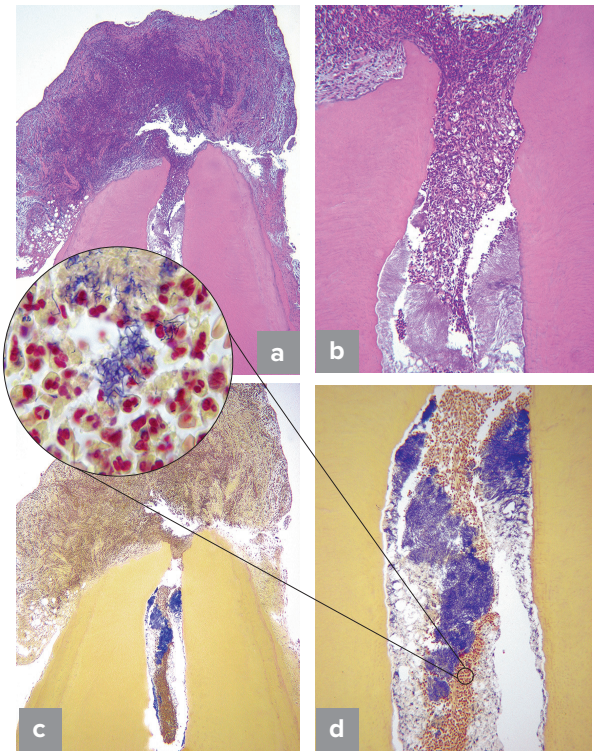
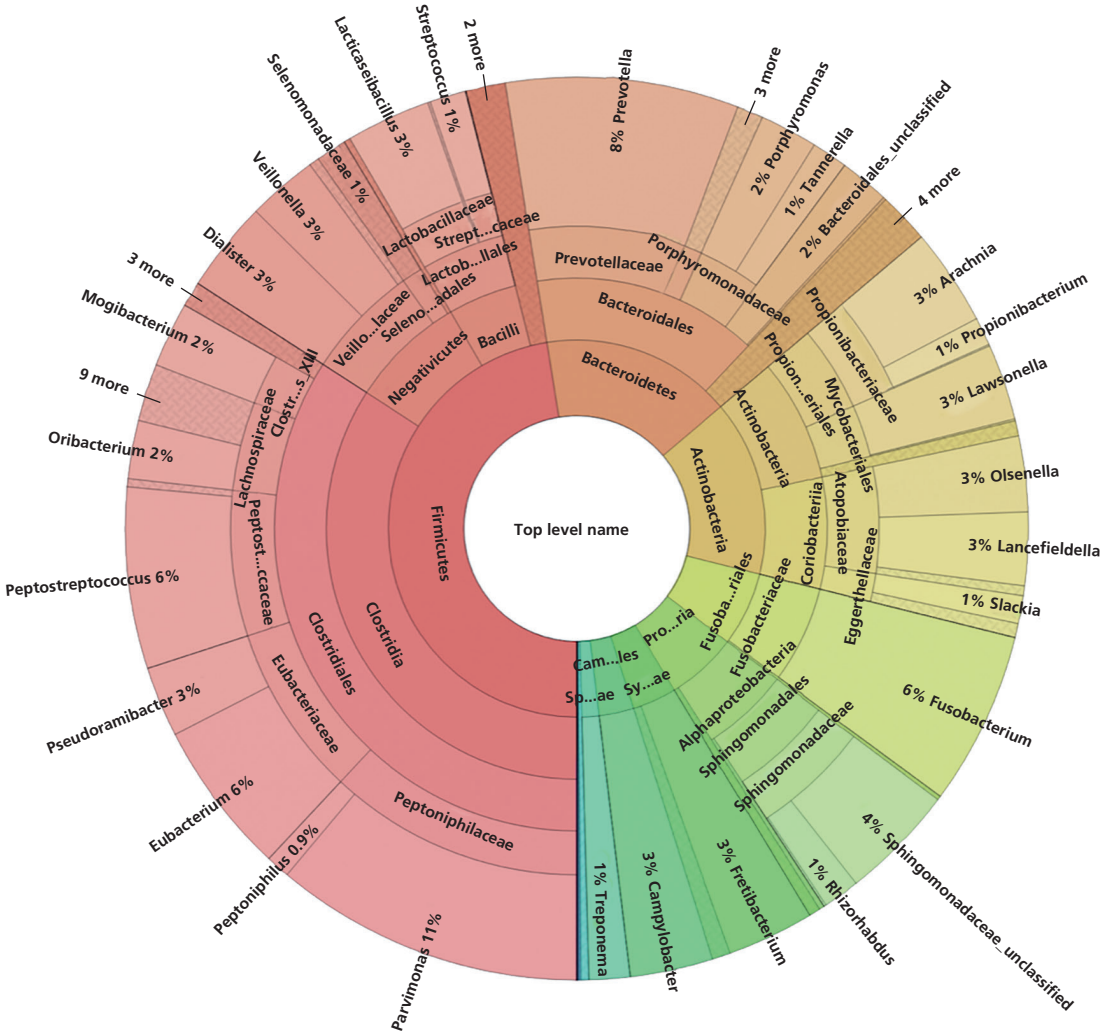



◀ **FIG 6-6** Apex of the mesiobuccal root of a maxillary molar with apical periodontitis: section passing through the main canal (h&e, Brown and Brenn staining ×25). *(inset)* Magnification of the most apical portion of the root canal (×100). *(a and b)* Note the inflammatory tissue with masses of amorphous substance (bacterial colonies) coronally and the marked resorption on the root canal walls and on the external radicular aspect. *(c and d)* Note the bacterial agglomerates surrounded by a concentration of inflammatory cells and the presence of filamentous bacterial forms surrounded by polymorphonuclear neutrophils. (Reprinted with permission from Ricucci and Siqueira 2013.)

▼ **FIG 6-7** Krona chart showing the bacterial taxonomic units of teeth with primary endodontic infections. (Reprinted with permission from Ordinola-Zapata et al 2023.)



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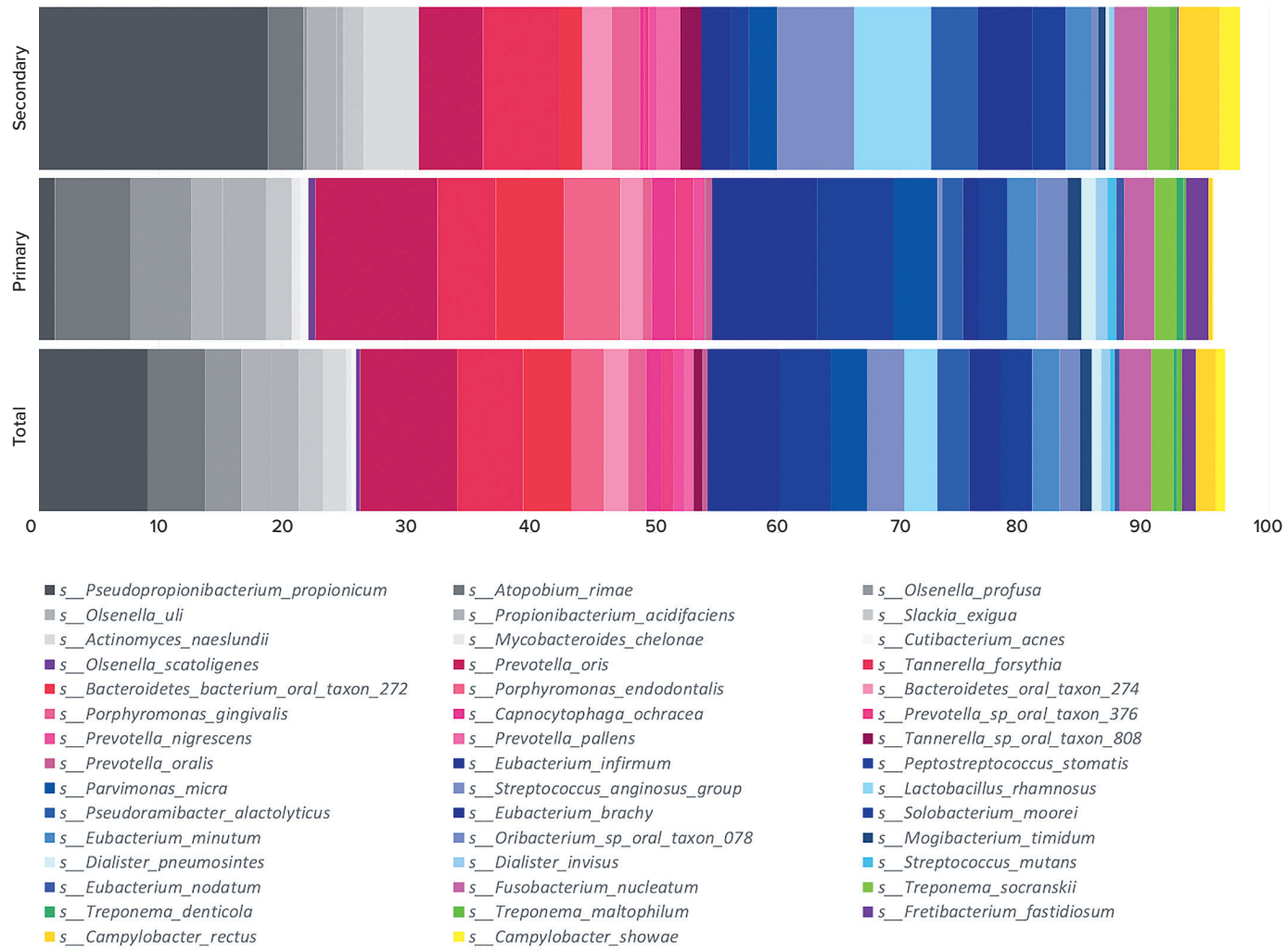


▲ **FIG 6-8** Bacterial species with > 0.3% relative abundance found in cases of primary and secondary endodontic infections obtained by shotgun sequencing. Actinobacteria, Bacteroidetes, and Firmicutes are represented with the *gray*, *red*, and *blue* scales, respectively. (Reprinted with permission from Ordinola-Zapata et al 2024.)

a causal link between an infectious disease and biofilms (Parsek and Singh, Hall-Stoodley and Stoodley):

1. The infecting bacteria are adhered to or associated with a surface.
2. Direct examination of infected tissue shows bacteria forming populations encased in an extracellular matrix.
3. The infection is generally confined to a particular site, and although dissemination may occur, it is a secondary event.
4. The infection is difficult or impossible to eradicate with antibiotics even though the responsible microorganisms are susceptible to antimicrobials in the planktonic stage.
5. There is ineffective host clearance evidenced by the location of bacterial colonies in discrete areas in the host tissue associated with host inflammatory cells (Ricucci and Siqueira 2010).

Ecologic factors can play a crucial role in the formation of biofilms in root canals and will determine their endurance to root canal treatment procedures (Chavez de Paz and Marsh). The ecologic processes occurring after microbial invasion in root canals can be subdivided into three steps: *(1)* habitat filter, *(2)* selection of resistant bacteria, and *(3)* resilience or posttreatment recomposition of the microflora (see "Secondary/persistent endodontic infections").

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## Habitat filters: Inflammation and necrosis

The oral ecosystem harbors one of the richest microbiomes in the human body, by far dominated by bacteria, although archaea, fungi, viruses, and protozoa may also be found (Avila et al, Costalonga and Herzberg). At least 774 species-level bacterial taxa have been cataloged in the human oral microbiome database; among them, 58% are officially named and 26% are known only as uncultivated phylotypes (Dewhirst et al, Human Oral Microbiome Database 2023). Because root canal infections most often occur as a sequel to caries or trauma, the bacterial communities that initially colonize the pulpal space result from an ecologic selection of the oral microbiota (Hsiao et al, Zehnder et al).

In a classic manuscript, Sundqvist (1994) reviewed the factors involved in the ecology of the root canal. The presence of numerous anaerobic species including *Fusobacterium*, *Parvimonas*, *Prevotella*, *Porphyromonas*, and *Treponema*, among others, could be due to numerous reasons: *(1)* the bacteria share a similar ecologic niche and growth requirements; *(2)* there is a possible synergistic interaction (eg, they may produce metabolic byproducts or enzymes that complement each other's growth, leading to a positive association); *(3)* there is adherence and biofilm formation; and *(4)* the bacteria have shared virulence factors.

When bacteria invade the pulp chamber, they will first encounter an environment filled with inflammatory mediators regulated primarily by signaling and activation of neutrophils at the site (Sundqvist 1992a, Sundqvist 1992b, Sundqvist 1994, Ricucci and Siqueira 2010). Bacteria will primarily adapt to and survive in this inflamed environment predominated by the production of reactive oxygen intermediates. These oxidative bursts caused by defending neutrophils will result in a massive killing of bacteria (Chávez de Paz and Marsh). Successful invaders will be those that can adapt to this neutrophil oxidative environment. After a while, the remaining pulpal tissue will necrotize, and another crucial change in the environment will take place. In necrosis, nutritional demands will determine the ecologic scenario, limiting the growth of one bacterial species in relation to others (Sundqvist 1992b).

The presence of byproducts resulting from the amino acid and peptide metabolism of fastidious microorganisms explains the differences between the microbial composition of the root canal space and that of other oral habitats, such as saliva or caries. These byproducts originate from connective tissue breakdown, serum glycoproteins, and inflammatory exudate (Sundqvist 1994). In the necrotic environment, a large network of metabolites and enzymatic reactions will take place (see Fig 6-9). Complex metabolic networks will filter and select species that will comply with the restricted nutritional demands of the root canal environment (Sundqvist 1992b, Sundqvist 1994, Figdor et al). In microbial communities, the presence of multiple microbial species or functional genes that perform similar or overlapping functions is called *functional redundancy*. This mechanism can provide resilience and resistance to environmental changes or disturbances, ensuring the continuity and stability of ecosystem functions even if some species or genes are lost (Siqueira and Rôças 2009a). The proteolytic enzymatic activity of root canal bacteria is an example of this.

Oxygen levels in the pulpal ecosystem may also be important in root canal microbial community selection as well as in determining functional interactions and spatial structures (Loesche, Loesche et al 1983). Previous studies on the dynamics of endodontic infections have shown that the relative proportions of anaerobic bacteria increase with time and that the facultative anaerobic bacteria are outnumbered when the canals have been infected for 3 months or more (Möller et al, Fabricius et al 2006). Also, when combinations of bacterial strains originally isolated from an infected root canal were inoculated into noninfected root canals, a dominance of anaerobic bacteria was established (Fabricius et al 1982a, Fabricius et al 1982b). Clostridiales (*Parvimonas*, *Peptostreptococcus*, *Eubacterium*, and *Pseudoramibacter*) produce butyrate, $CO_2$, and hydrogen ($H_2$) by anaerobic fermentation, which decreases the pH of the environment. In the infected root canal environment, oxygen concentration gradients range from low to total anoxia. Although oxygen gradients can be relatively constant over time, oxygen appears to be an important ecologic factor in the root canal environment, promoting the proliferation of anaerobic bacteria (Sundqvist 1992b, Sundqvist 1994).

In general, the root canal milieu may be viewed as a distinct biologic niche with possibly distinct nutritional requirements compared to the dental plaque or saliva environments. Overall root canal microbiome composition is likely shaped by the enzymatic capability of the microbial community in response to nutrients in the root canal milieu (Fig 6-9).

## Endodontic infections and symptoms

Symptomatic endodontic infections and their complex associations with black-pigmented bacteroides were studied during the 1980s. Sundqvist et al (1989) revealed that *Bacteroides intermedius* (*Prevotella intermedia*), *Bacteroides gingivalis* (*Porphyromonas gingivalis*), and *Bacteroides endodontalis* (*P endodontalis*) played a crucial role in acute apical abscesses. Their study of 72 cases revealed that these bacteria were present in 30% of the cases, with 16 cases developing purulent discharge while 6 remained


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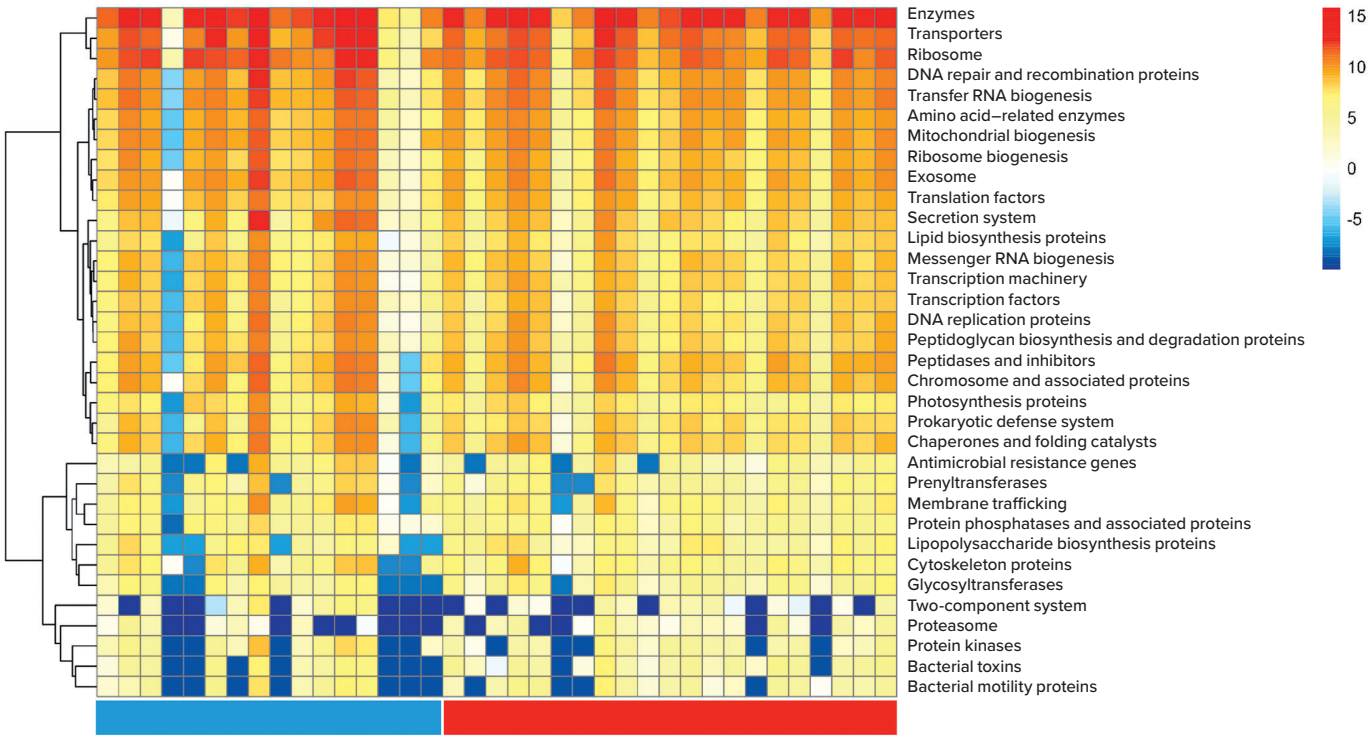
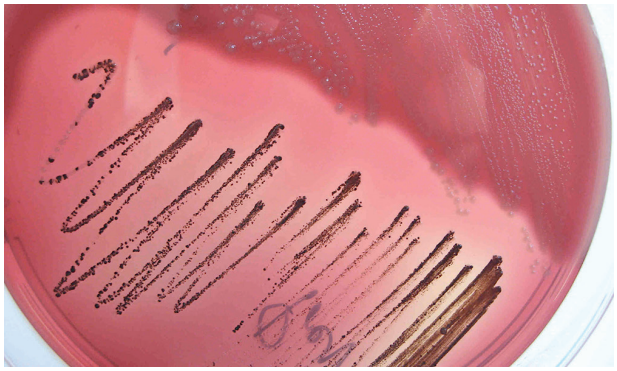



▲ **FIG 6-9** The root canal microbiome can be considered a mixture of potential enzymatic activities. This heat map depicts protein families according to the KEGG BRITE classification (shotgun sequencing). *Red* indicates samples from primary endodontic infections, and *blue* indicates samples from secondary/persistent endodontic infections. The top 25 genes were involved with genetic information (ribosomal proteins) and signaling and cellular activities (ABC transporters), as well as the iron complex transport system. The most prevalent genes in metabolism and enzymatic processes were oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases, and translocases. The second- and third-most prevalent genes in this category were peptidases and amino acid–related enzymes, respectively. (Reprinted with permission from Ordinola-Zapata et al 2024.)

asymptomatic. The pathogenicity and virulence of black-pigmented gram-negative anaerobes have been proved in in vitro and animal experiments (Sundqvist 1993). Previous studies have revealed that capsular structures among these microorganisms are more resistant to phagocytosis in vitro; their byproducts include toxic elements including sulfuric acid, indole, and ammonia. Studies found that *Prevotella* and *Porphyromonas* spp have a high ability to form hydrogen sulfide ($H_2S$) in human serum (Persson et al). On the other hand, Haapasalo et al discovered that black-pigmented bacteroides were found in both symptomatic and asymptomatic infections (Fig 6-10). There were also several symptomatic cases that did not harbor black-pigmented bacteroides.



▲ **FIG 6-10** Black-pigmented bacteroides (*Prevotella* and *Porphyromonas* spp) have been implicated in periodontal and endodontic infections. The hemolytic ability of this strain can be observed.

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Gomes et al (1996a) aimed to determine if any combination of bacteria was associated with endodontic signs or symptoms. Samples taken from 70 cases were cultured. The authors revealed that 65 different species were identified, and 10 cases without detectable bacteria presented with pain, tenderness, swelling, sinus tract, wet canal (hemorrhagic/purulent exudates), or periapical radiolucency as clinical features. Obligate anaerobes accounted for 64% of the total species isolated and 70.3% of all symptomatic teeth. Many of these anaerobes were *Prevotella* and *Peptostreptococcus* spp. These bacteria, in addition to *Eubacterium* spp, also accounted for cases that presented with swelling and wet canals. Significant associations were noted between symptoms of pain, swelling, and/or wet canals and particular pairings of *Prevotella* spp, *Peptostreptococcus* spp, and *Eubacterium* spp.

De Brito et al investigated the microbial communities of endodontic infections at their apical portion by 16S rRNA sequencing as well as their association with clinical symptoms. Samples were collected from the apical third of 15 teeth. Operational taxonomic units (OTUs) were assigned to 10 bacterial phyla, led by Bacteroidetes (51.2%) and Firmicutes (27.1%), and 94 genera were represented primarily by *Prevotella* (17.9%) and *Bacteroidaceae* G-1 (14.3%). Symptomatic teeth were associated with higher levels of *Porphyromonas* and *Prevotella* spp. *P endodontalis* and *Prevotella oris* were present in both cores (symptomatic and asymptomatic).

Although the published literature allows us to have a better understanding of the microbial composition of endodontic infections, it is difficult to establish causality between taxonomy-based datasets and specific clinical scenarios (ie, presence of pain or percussion sensitivity). Thus, NGS and other taxonomy-based inferences can only be considered as a first screening tool to assume microbiome function (Ordinola-Zapata et al 2023). It is important to note that patients' individual variations can mask symptom-related signatures, and endodontic symptoms can be influenced by several factors including the longevity of the infection, bacterial load, expression of virulence factors, host resistance, genetic predisposition, and the root canal microenvironment at different stages of pulpal degeneration. It is also accepted that bacteria from the same species can present different pathogenic traits (Georgiades and Raoult). For example, different strains of the periodontal pathogen *P gingivalis* have a range of virulence potentials (Tribble and Lamont). *P gingivalis* strains differ in capsular characteristics, proteases, and microbe-associated molecular patterns (MAMPs), which can activate proinflammatory and osteoclastogenic mediators (Graves et al). It is also important to note that previous studies that associated symptoms with the composition of the root canal system were observational in nature and only established a correlation.

## Primary endodontic infections associated with periodontal disease

Endodontic-periodontal lesions are characterized by the pathologic and inflammatory interaction between the pulpal and periodontal tissues through open structures such as apical foramina, lateral canals, accessory canals, and dentinal tubules. Common clinical and radiographic features include deep periodontal pockets reaching or extending to the apex, altered response to pulp vitality tests, bone resorption in the apical or furcation region, spontaneous pain, pain on palpation, tenderness to percussion, purulent exudate, tooth mobility, sinus tract, and changes in crown and gingival color (Gomes et al 2015, Gomes et al 2022).

Lopes et al performed a systematic analysis using state-of-the-art sequence data to assess the microbial composition of 12 infected root canals and their associated periodontal pockets to verify the possibility of cross-infection between these sites. The number of species in the periodontal pocket was higher than that in root canals, and there was a predominance of obligate anaerobes and gram-negative bacteria. In the root canals, the genera *Enterococcus*, *Parvimonas*, and *Stomatobaculum* predominated. The periodontal pockets included these genera as well as *Peptostreptococcus* and *Mogibacterium*. The root canal microbiota was less complex and diverse than that of periodontal pockets. In a subsequent study, Gomes et al (2022) confirmed the predominance of Firmicutes, Proteobacteria, and Actinobacteria phyla in 10 root canals of teeth with combined endodontic-periodontal diseases. The most abundant genera were *Parvimonas*, *Enterococcus*, *Stomatobaculum*, *Peptoniphilaceae*, and *Peptostreptococcus*. *P micra*, *Enterococcus* spp, and *Stomatobaculum* spp were the predominant species at baseline.

## Secondary/persistent endodontic infections

Endodontic treatment plays a crucial role in the elimination of bacteria associated with root canal infections. It not only targets and reduces the bacterial load but also disrupts the ecologic balance within the root canal system (Sundqvist 1992b). The microorganisms found in previously treated teeth are composed of a more restricted group of bacteria when compared to primary endodontic infections. These variations are both quantitative and qualitative in nature (Molander et al). Posttreatment apical periodontitis is associated with bacteria not disturbed by the cleaning and shaping


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protocol (persistent infection) or introduced during or after root canal treatment (secondary infection).

## Selection of resistant bacteria

The mechanical and antimicrobial actions of root canal treatment procedures are intended to eliminate bacteria in infected root canals. The mechanisms of bacterial adaptation and survival include phenotypic changes in the form of rapid protein or enzymatic expression. These mechanisms triggered by the presence of antimicrobials have been widely studied in several areas of medicine (Landini and Valle). However, the mechanisms underlying adaptation to the exposure to endodontic antimicrobials remain unclear (Svensäter and Bergenholtz, Chavez de Paz 2007, Ricucci and Siqueira 2010).

The mechanisms inherent to the survival of biofilm bacteria to antimicrobials are divided into two main categories: resistance and tolerance. Resistance is primarily determined by the genetic fitness of bacteria to withstand the presence of antimicrobials. The activation of genes when antimicrobials are present in the environment represents these mechanisms. For example, *Enterococcus faecalis* has an intrinsic resistance to alkaline antimicrobials exerted by an efficient cell membrane Na-H antiporter system. Other intrinsic mechanisms of resistance include the limited penetration of antimicrobial molecules through the biofilm matrix, modification of cell receptors, and inactivation of the antibiotic properties (Chavez de Paz et al 2007).

Tolerance is an acquired ability "learned" by bacteria in the presence of antimicrobials. This is characterized by temporary phenotypic alterations in the cell that allow bacteria to tolerate antimicrobials. The mechanisms of tolerance do not act directly against antimicrobials to neutralize the antimicrobial effect but occur as a cascade of intracellular events (Ciofu et al). Although most of the mechanisms of adaptation are not yet known, some of these mechanisms have been studied in biofilm bacteria in the presence of antimicrobials or other environmental changes such as nutrient deprivation. For example, when the environment is deprived of nutrients, there is a metabolic downregulation in the cell that limits the energy requirements for bacterial survival (Stewart). In the same fashion, the effect caused by the presence of antimicrobials in the environment induces physiologic changes in the cells by regulating specific genes (Stewart and Bjarnsholt).

## Resilience: Posttreatment recomposition of the microflora

Biofilms established in the infected pulp canal space are a true concern for endodontics, as bacteria within biofilms become increasingly resistant to antimicrobials and host defenses (Chavez de Paz et al 2010). As these biofilm communities form on root canal irregularities that are beyond the reach of mechanical removal and the clinical effect of antimicrobials (Nair et al 2005), host-derived proteins from remaining necrotic tissues and bacterially produced adhesive substances will provide the necessary conditions for such microbes to survive in the treated root canal space (Chavez de Paz and Marsh).

Several studies have shown that, although highly effective, endodontic treatment procedures are not 100% effective in removing biofilm bacteria (Keskin et al, Sanchez-Sanhueza et al). It is considered feasible that a very small portion of the original endodontic microflora will survive and readapt after treatment. The persistence of remaining bacteria inside the root canal system can be affected by the anatomical complexity of the apical third, the presence of apical root resorption, or insufficient disinfection procedures.

The mechanisms of readaptation exerted by persistent biofilm bacteria in the root canal are highly unknown; however, it is likely that persisting organisms play a role in treatment failures (Engström et al, Möller, Moller et al, Molander et al, Fabricius et al 2006). Persistent bacteria following root canal treatment are mainly commensal bacteria from the oral cavity that have adapted to endodontic antimicrobials. The persistent endodontic microflora has been characterized to be dominated by gram-positive facultative anaerobes (Engström et al, Möller, Moller et al, Molander et al, Fabricius et al 2006). Their persistence is attributed to the hardiness of their cell wall structure and their physiologic plasticity that allows them to effectively adapt and tolerate harsh environmental conditions (Nawrocki et al, Assoni et al). Only a few studies have shown traits of the mechanisms regulating the adaptation of root canal bacteria to antimicrobials (Chavez de Paz et al 2007, Chavez de Paz et al 2010, Chavez de Paz 2012). Antimicrobials generally stress the root canal environment, forcing bacteria to adapt their physiology to survive.

Studies have revealed that endodontic therapy can also alter the environmental conditions inside the root canals, leading to the elimination of certain bacteria that may be necessary for the growth of fastidious bacteria. Additionally, residual nutrients within the root canal system provide a favorable environment for the growth of remaining microorganisms. Notably, certain bacteria may display "host compatibility," meaning they can coexist with the

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host without producing reinfection or disease (Gomes et al 1996b). Therefore, the success of treatment for oral infections should be considered in the context of the complex interplay between microbial factors and host factors in the development of disease.

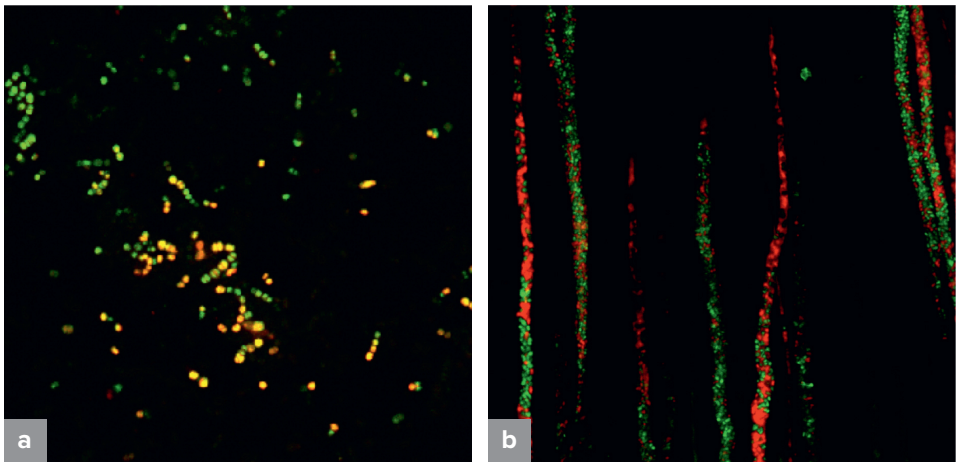
## Secondary contamination

Coronal leakage and bacteria introduced during the root canal treatment are causes of secondary contamination. The presence of coronal microleakage during or after endodontic treatment can alter the nutritional conditions within the root canals and contribute to bacterial growth of facultative bacteria such as *Streptococcus*, *Staphylococcus*, *Enterobacter*, *Klebsiella*, and *Pseudomonas* spp (Molander et al, Sundqvist et al 1998, Siqueira 2002, Pinheiro et al).

Zahran et al revealed that around half of the rubber dam surfaces they investigated were contaminated with significant bacterial loads at the time of obturation, and bacteria were also detected in 20% to 30% of gloves, endodontic instruments, and rulers prior to obturation. Thus, involuntary bacterial contamination of root canals before obturation is still a risk even under current endodontic best practices. In this regard, it is essential to emphasize the presence of Proteobacteria in research that examines the microbial composition of previously filled teeth. Keskin et al found that Proteobacteria represented the most abundant phylum in secondary infections. Similarly, Anderson et al and Sanchez-Sanhueza et al reported the relative abundance of this group in 26% and 50% of the total OTUs, respectively. This data is in accordance with Siqueira et al (2016), who found that Proteobacteria was the most abundant phylum (46%) in well-treated endodontic cases, with *Pseudomonas* as the genus most associated with secondary infections (Siqueira et al 2016, Sanchez-Sanhueza et al). The evidence also appears to show no differences related to the radiographic quality of the obturation and the presence of Proteobacteria in previously treated teeth (Anderson et al, Siqueira et al 2016, Sanchez-Sanhueza et al).

## Microbial diversity in secondary/persistent endodontic infections

Using the culture technique, Molander et al revealed that one or two strains of bacterial species were most commonly isolated from root-filled teeth with apical periodontitis (85%), with *Enterococcus* being the most prevalent (69%). *E faecalis,* a facultative gram-positive bacterium, is able to survive in an environment with poor nutrient availability and minimal interaction with other species. Numerous studies using culture or molecular techniques confirmed that *E faecalis* was the bacterial species found most frequently in previously treated teeth (Molander et al, Sundqvist et al 1998, Pinheiro et al, Gomes et al 2008, Rôças and Siqueira 2004, Siqueira and Rôças 2004, Anderson et al, Bouillaguet et al). It presents different mechanisms of virulence and resistance compared to other species, and as a result it can be difficult to eliminate it from the root canals. An important collagen-binding protein of the microbial surface components of *E faecalis* is Ace (*E faecalis* collagen adhesion), which is related to the process of invasion and adhesion of dentinal tubules (Fig 6-11) and collagen, respectively, when human serum is present (Molander et al, Sundqvist et al 1998, Signoretto et al, Love 2001, Siqueira 2002, Pinheiro et al, Fisher and Phillips). Evans et al observed




◀ **FIG 6-11** *(a and b)* CLSM photomicrographs showing *E faecalis* cells invading dentinal tubules.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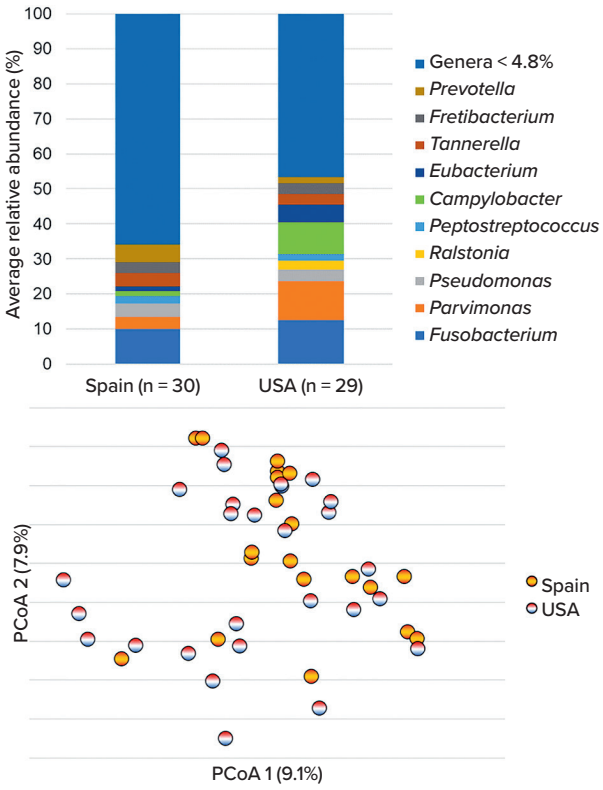
that *E faecalis* was resistant to calcium hydroxide medication at a pH of 11.1 and that cellular survivability was associated with the presence of a proton pump in this microorganism.

The extensive data on the association of *E faecalis* with endodontic failures has become controversial during recent years because of the low abundance of this species in more recent molecular studies (Hong et al, Anderson et al, Siqueira et al 2016, Keskin et al, Sun et al, Ordinola-Zapata et al 2023). Furthermore, studies that obtained samples from the apical third by cryopulverization were not able to identify these bacteria in a significant number of cases.

Microbial diversity considers the richness and evenness of a microbial population. Five studies have evaluated the microbial diversity of primary and secondary endodontic infections using NGS methods (Hong et al, Tzanetakis et al, Keskin et al, Bouillaguet et al, Ordinola-Zapata et al 2003). Bouillaguet et al and Ordinola-Zapata et al (2023) found that primary infections are more diverse than secondary/persistent infections. In contrast, Hong et al and Keskin et al could not find differences between primary and secondary infections, and Tzanetakis et al reported a higher diversity in secondary/persistent infections.

Gram-positive rods have also been identified in samples obtained after root canal treatment, including *Olsenella uli*, *Lactobacillus paracasei*, and *Propionibacterium propionicum* (Chavez de Paz 2012). A high relative abundance of *Fusobacterium* spp in primary infections is not an uncommon finding in more recent molecular studies (Siqueira et al 2016, Bouillaguet et al, Zandi, Manoharan et al). *Fusobacterium* has been reported to be the dominant genus in previously treated teeth (Hong et al, Keskin et al, Tzanetakis et al, Ordinola-Zapata et al 2023). A recent study (Arias-Moliz et al) revealed that the overall microbiome diversity in previously treated teeth was similar in samples obtained in Spain and the United States. *Fusobacterium* was the most abundant genus in both geographic locations (100% and 88% prevalence, respectively), followed by *Parvimonas*, *Campylobacter*, *Arachnia*, *Pseudoramibacter*, *Streptococcus*, *Prevotella*, *Eubacterium*, *Fretibacterium*, and *Tannerella* (Fig 6-12). Overall, NGS data suggests that persistent/secondary infections are dominated by *(1) Pseudomonas* spp (Siqueira et al 2016, Sanchez-Sanhueza et al), *(2)* a mixed infection dominated by anaerobic species (Tzanetakis et al, Keskin et al, Arias-Moliz et al, Ordinola-Zapata et al 2023), or *(3)* gram-positive facultative bacteria including *E faecalis* (Molander et al, Sundqvist et al 1998, Pinheiro et al). A significant number of manuscripts in the field could not confirm causation between the origin of microorganisms and the




▲ **FIG 6-12** Microbiome of previously treated teeth showing the relative abundance of bacterial genera in samples from Spain and the United States. Genera reflecting a mean of < 3.4% of sequence reads among all samples were consolidated. Principal coordinate analysis (PCoA) showed no significant difference between the two geographic locations. (Reprinted with permission from Arias-Moliz et al.)

persistence of apical periodontitis, which may explain these variances (ie, secondary contamination, inadequate treatment, or coronal leakage). The taxonomic abundance could also be affected by the time between root canal treatment and the identification of treatment failure. Figures 6-13 and 6-14 show the presence of bacteria in treated root canals.

## Extraradicular infection

In contrast to periodontal disease in which microbial invasion of periodontal cells is common (Tribble and Lamont), apical granulomatous tissue rarely contains bacteria. The genetic predisposition of the host (Morsani et al), environmental exposure (secondary contamination), and microbiome composition can affect disease susceptibility. It is commonly accepted that the dynamic of host-associated microbial communities is made up of static functional units like commensal bacteria that might cause harm to the host (pathobionts). However, within-host evolution has proven

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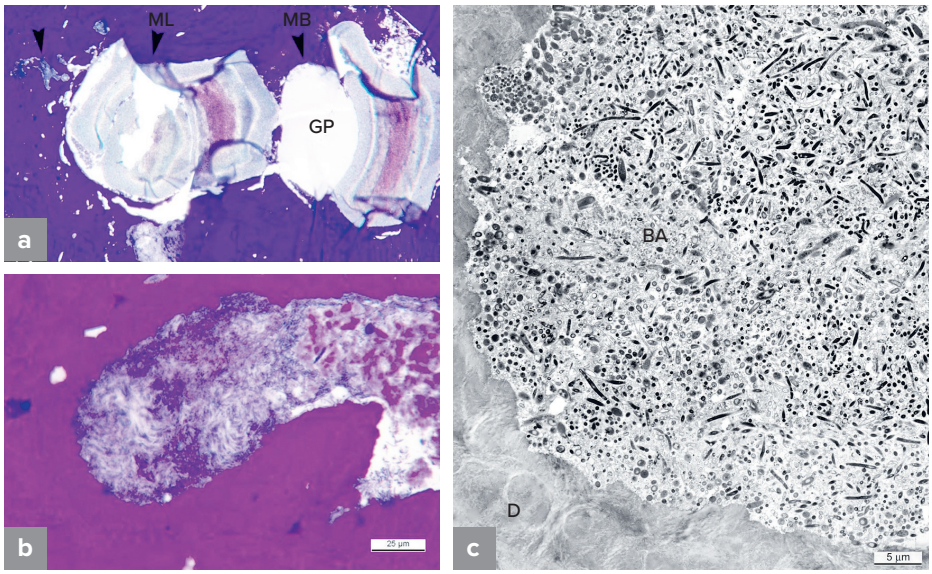


**FIG 6-13** *(a)* Photomicrograph of a transverse section through the apical portion of the mesial root of an extracted tooth. *(b)* A large accessory canal is clogged with bacteria. *(c)* Transmission electron microscopic view showing a bacterial mass in the noninstrumented necrotic accessory canal. Note the filamentous bacteria (BA) embedded in an extracellular matrix material. D = dentin; ML = mesiolingual; MB = mesiobuccal. (Original magnification ×3200; reprinted with permission from Nair et al 2005 and courtesy of Jorge Vera.)

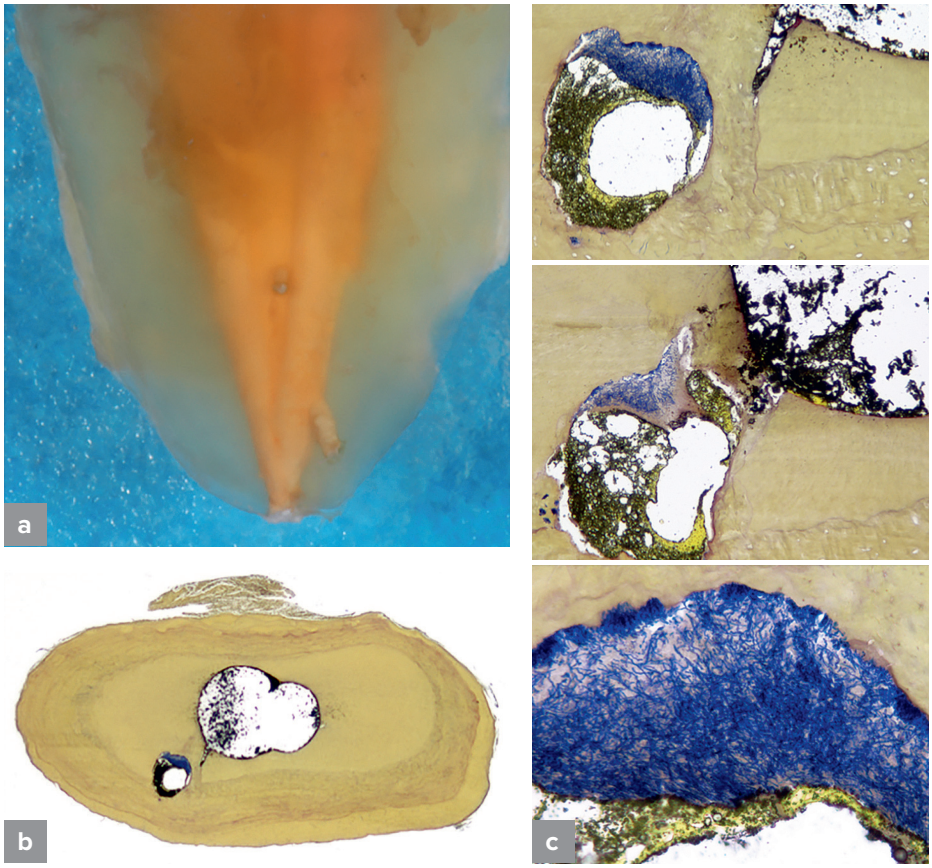


**FIG 6-14** *(a and b)* Mandibular left first molar with destructive carious process. The root canal in the mesial root was completed in one visit. In the distal view of the cleared specimen, apical ramifications "filled" by obturation material can be seen. *(c)* Sequence of sections encompassing the whole course of the most apical ramification. A thick bacterial biofilm with high density of filamentous forms is surrounded by obturation material. (Original magnification ×100 and ×400; reprinted with permission from Vera et al and courtesy of Jorge Vera.)



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that microbial strains may adapt and become more virulent throughout the course of a human lifetime. In fact, some bacterial species can modify their cell wall structure to evade detection and clearance by the immune system (Yang et al).

Extraradicular infections include acute apical abscesses, colonization by extraradicular biofilms, and periapical actinomycosis (Siqueira 2002, Gomes and Herrera, Manoil et al). Periapical actinomycosis is a type of infection characterized by bacterial colonization of apical tissues, particularly by *Actinomyces* spp or *P propionicum*. While acute apical abscesses are typically resolved through endodontic treatment, cases involving extraradicular biofilms or actinomycosis often require surgical root canal treatment (Gomes and Herrera, Siqueira and Rôças 2022). Multiple authors have concluded that the incidence of extraradicular biofilms is low compared to intraradicular biofilms (Sundqvist and Reuterving, Tronstad et al 1987, Sjögren et al 1988, Ricucci et al 2010, Ricucci et al 2015, Ricucci et al 2018).

One of the first reports on extraradicular infection was described by Tronstad et al (1990). The authors microscopically examined the surface of root tips from 10 patients with nonhealing lesions that underwent root-end surgery; the samples were studied under the scanning electron microscope. The results revealed that all samples contained bacteria, many of which were anaerobic. The root tip surfaces were covered by a variety of bacterial forms in a biofilm. In the same year, Nair et al (1990) used light and electron microscopy to assess the presence or absence of microorganisms within previously treated periapical lesions 4 to 10 years following endodontic treatment. During endodontic surgery, rectangular block sections of the periradicular tissues were retrieved. Four of the nine lesions had bacteria present, and two had fungus.

Refractory endodontic infections frequently indicate that microorganisms have survived nonsurgical treatment and continue to produce periapical inflammation. Ricucci et al (2005) identified two cases of refractory disease with calculus-like deposits harboring bacterial aggregations on resected root tips. The authors hypothesized that minerals are supplied via a patent sinus tract or periapical inflammatory exudate and found that these bacteria can play a role in refractory periapical disease.

Although immune cells and bacterial pathogenicity are considered important variables that influence the spread and survival of microorganisms in the periapical tissues, the processes underlying microbial invasion and persistence in these areas remain poorly understood. In an animal experiment, Walton and Arjmard revealed that bacterial invasion in endodontic infections was restricted to the root canal space in 16 out of 18 experimental cases. The authors suggested that the immune response may effectively eliminate bacteria and their metabolites from apical tissues. Another study that employed bacteria-specific staining (Brown and Brenn) found that only 6% of previously treated teeth and teeth with primary infections had extraradicular bacterial biofilms (Ricucci and Siqueira 2010). These infections were invariably linked to an intraradicular colonization.

Some gram-positive bacteria, especially *Actinomyces* and *Propionibacterium* spp, can survive in the periapical tissues, forming extraradicular biofilms. Nair et al (2008) investigated the ability of *Actinomyces* spp to establish infections in subcutaneous murine tissue cages. The authors discovered that the key to *Actinomyces israelii*'s pathogenicity was its capacity to form compact colonies in host tissues, allowing it to evade host defenses and cause disease. Actinomycosis can be a recurrent complication when the periapical tissues become infected and nonsurgical root canal treatment fails to promote the healing of apical tissues (Siqueira 2003). *Arachnia* is indistinguishable from *Actinomyces* on histologic slides (Brock et al). *Arachnia* can be considered an important agent of suppurative lesions with or without the production of typical actinomycotic sulfur granules (Brock et al, Happonen, Happonen et al). The self-flocculation property of this microorganism allows it to increase its biomass, which may enhance its tolerance to the periapical tissues. Long-standing infections by *Actinomyces* or *Propionibacterium* spp are characterized by cortical bone destruction, yellow "sulfur crystals" in purulent drainage, and sinus tract development (Siqueira 2003). Happonen summarized 16 cases in which actinomycosis was found in the periapical tissues. In all but one case, surgical root excision and curettage of the periapical bone produced satisfactory periapical healing. Actinomycosis responds to β-lactam antibiotics, but the only definitive treatment is surgical root-end resection and curettage of the tissues involved (Siqueira 2003).

Sunde et al studied 39 periapical lesions using fluorescence in-situ hybridization with specific probes. The authors revealed the presence of numerous morphotypes including rods, spirochaetes, and cocci, in some cases forming microcolonies. *P gingivalis*, *P intermedia*, *Tannerella forsythia*, and *Treponema* spp were detected in different layers of periapical lesions of root-filled teeth. In another study, Ricucci et al (2018) performed a histobacteriologic analysis of apical root and lesion specimens taken from individuals with chronic apical abscesses and sinus tracts. They found that 83% of samples had an extraradicular component of infection that was a continuum of an intraradicular infection (Fig 6-15). Teeth that had chronic apical abscesses and sinus tracts displayed a very complicated infectious pattern in the apical root canal system and periapical lesion, with biofilms taking up the majority of

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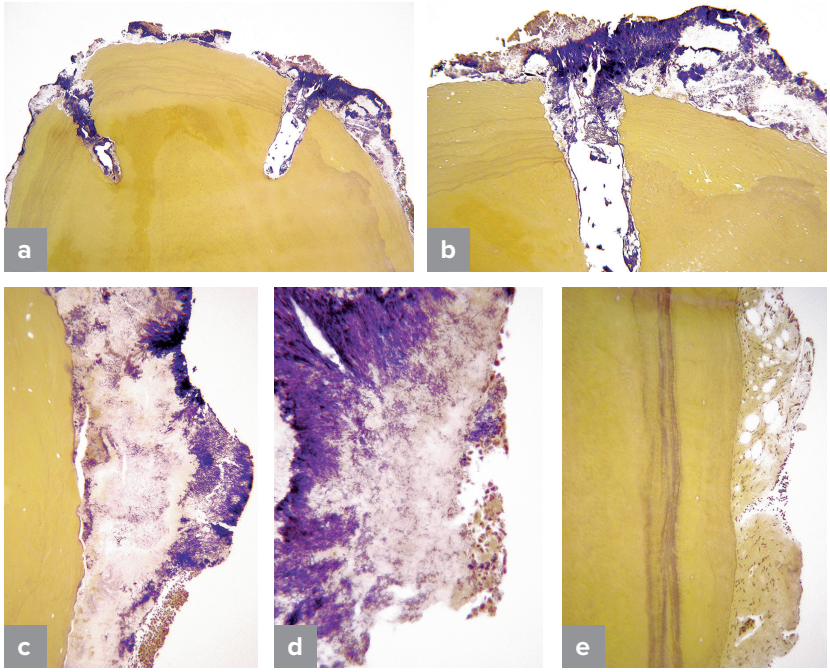


◀ **FIG 6-15** *(a to e)* Histology obtained from the apical third of an extracted maxillary left premolar with exposure of the root canal space. Repeated abscesses were recorded in this case. At the time of extraction, there was a sinus tract on the buccal mucosa. Calculus formation has affected the apical root surface. Note how the main canal splits close to the apex. The accessory apical anatomy appears to be filled with an amorphous structure with abundant bacteria. This structure continues on the external radicular aspect. (Reprinted with permission from Ricucci and Siqueira 2013.)

the space (Ricucci et al 2018, Siqueira and Rôças 2022). In a recent study, Pérez-Carrasco et al revealed the presence of numerous oral bacteria in periapical lesions of previously treated teeth using NGS. This included the following genera: *Fusobacterium*, *Porphyromonas*, *Streptococcus*, *Pseudomonas*, *Fretibacterium*, and *Tannerella*, among others. In the existing literature, only a limited number of cases have been documented where extraradicular infection was not visibly linked to intraradicular bacteria and was identified as the suspected cause of persistent post-treatment disease. These cases could only be resolved through surgical intervention (Ricucci et al 2015, Ricucci et al 2018, Siqueira and Rôças 2022).

Bacterial invasion of eukaryotic cells in the connective tissue can be found in periodontal disease (Tribble and Lamont). Numerous oral and non-oral bacteria can survive within epithelial cells and damage the attachment apparatus (Rudney et al, Tribble and Lamont, Graves et al). The invasion capacity also helps microorganisms to evade the innate immune system (Tribble and Lamont). A WGS study of the root canal microbiome revealed the presence of the gene internalin A in a prevalent endodontic pathogen, *P micra* (Ordinola-Zapata et al 2023). Internalin A is a protein commonly used by *Listeria monocytogenes* to adhere and invade nonphagocytic cells. Oral bacteria such as *P gingivalis*, *P intermedia*, *Tannerella forsythia*, and *Treponema denticola* can also present genes encoding internalin homologs or have intracellular survival capabilities (Duran-Pinedo et al). Residence within host cells provides bacteria with nutrition, avoiding clearance by immune mechanisms (Tribble and Lamont). This mechanism can explain the results of studies that were able to find evidence of microbial colonization in the periapical tissues, for example Sunde et al, Ricucci et al (2018), and Pérez-Carrasco et al.

## Acute apical abscess

As previously stated, polymicrobial endodontic infections can spread locally and systemically if the immunocompetence is compromised or the bacterial virulence increases. The most prevalent form of extraradicular infection is the acute apical abscess (AAA). AAA is an inflammatory response to pulpal infection and necrosis characterized by rapid onset, pain, tenderness upon percussion, pus formation, and swelling of the surrounding tissues (American Association of Endodontists). In the absence of early treatment, the presence of soft tissue edema may pose life-threatening concerns due to the infection's spread through the bone and other fascial spaces (Montagner et al). In many cases, an AAA may not present radiographic evidence of periradicular bone resorption. In cases where a periapical radiolucency is present, the abscess often results from the exacerbation of a preexisting asymptomatic apical periodontitis.

The microbial communities present in AAA cases are highly diverse, with a predominance of anaerobic microorganisms (approximately 90% of the isolates), including the


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gram-negative bacilli *Fusobacterium*, *Prevotella*, *Porphyromonas*, *Dialister*, and *Treponema* as well as gram-positive cocci (Siqueira et al 2001, De Sousa et al, Siqueira and Rôças 2009b, Montagner et al, Siqueira and Rôças 2013, Nóbrega et al 2016b, Rôças and Siqueira 2018, Gomes and Herrera). Van Dalen et al investigated the virulence of *P micros (P micra)* in combination with *Prevotella* spp to form abscesses in an animal model. These two genera (*Parvimonas* and *Prevotella*) exhibit pathogenic synergy. In this experiment, mixed infections compared with single-species infections were characterized by transmissibility of infection, larger abscesses, and higher bacterial counts in pus (Van Dalen et al, Araki et al).

In previous studies on the microbial composition of AAA, the focus has primarily been on analyzing the purulent collection (Siqueira and Rôças 2013, Siqueira et al 2016, Rôças and Siqueira 2018). However, some investigations have also examined root canal samples from teeth with AAAs (De Sousa et al 2003, Sousa et al, Nóbrega et al 2016a, Nóbrega et al 2016b, Gabrielli et al). Certain species have been found to predominate in both types of samples (root canal and pus), including *Dialister*, *Fusobacterium*, *Parvimonas*, *Porphyromonas*, *Prevotella*, *Treponema*, and *Streptococcus* spp (De Sousa et al, Siqueira and Rôças 2013, Montagner et al, Santos et al, Siqueira et al 2016, Nóbrega et al 2016a, Nóbrega et al 2016b, Gabrielli et al).

The treatment for AAA consists of the removal of the etiologic agent through root canal debridement and surgical drainage (Flynn). Antibiotics are important in the management of patients presenting signs and symptoms of systemic involvement, including cellulitis, malaise, and fever. This rule also applies to immunocompromised patients (Lockhart et al). The progression of an untreated AAA can lead to serious complications including Ludwig's angina (Srirompotong and Art-Smart), cavernous sinus thrombosis (Zachariades et al), respiratory tract involvement, mediastinitis (Pappa and Jones), sepsis, and death (Currie and Ho, Carter and Lowis). The clinical criteria that determine the severity of the infection include difficulty breathing and swallowing, trismus, involvement of fascial spaces, fever, and immunosuppression.

## Bacterial byproducts and other virulence factors

Studies utilizing the 16S rRNA amplicon approach (NGS) are particularly valuable for conducting functional prediction studies using methods such as PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; Langille et al), PICRUSt2 (Douglas et al), or Tax4Fun2 (Wemheuer et al). The aim is to estimate the functional profiles of bacteria using 16S rRNA data. Unlike NGS, shotgun metagenomic sequencing and RNA sequencing examine the whole DNA or RNA in a given sample (Wensel et al). This comprehensive sequencing approach allows for functional inference rather than mere prediction as observed in amplicon sequencing (Douglas et al, Logares et al, Vandamme et al). WGS studies enable the evaluation of metabolic pathways (Donovan et al, Kanehisa, Verberkmoes et al); the identification of novel genomes (Cabau et al, Hernandez et al, Lin et al 2017); and, importantly, the design of studies focused on intriguing genes such as mobile genetic elements (Martínez et al, Siefert), antibiotic-resistance genes (Carr et al, Crofts et al, Feng et al), or other virulence factors. This is particularly important because the type of bacteria that is most abundant is not always the one that is the most functionally dominant.

Microorganisms can cause damage to the host via a variety of mechanisms: *(1)* They can produce a synergistic interaction, for example by producing metabolic byproducts or enzymes that complement each other's growth, resulting in positive associations; *(2)* they can activate the innate and acquired immune systems; and *(3)* they can adhere and form biofilms (Siqueira and Rôças 2007). *Bacterial virulence* is a relative term that is contingent on the condition of a vulnerable host (Casadevall and Pirofski). The opposite side of the host-microbe connection consists of humans and their immunocompetence (Kumar). To explain the aggressiveness of oral opportunistic infections, a number of different mechanisms have been described. For example, through the metabolism of methionine and cysteine, the bacteria *Prevotella* and *Fusobacterium* can produce potentially harmful sulfur compounds (Foo et al). This process results in the production of $H_2S$, which is toxic to human cells and inhibits the growth of other bacteria. Carlsson et al studied the proteolytic ability of 37 oral species and revealed that *P micros (P micra)* and *F nucleatum* had a strong ability to digest tripeptides and produce $H_2S$. This activity is unique to opportunistic microorganisms such as *Escherichia coli* and *Pseudomonas aeruginosa*. *P micra* is the most prevalent cause of sepsis from gram-positive anaerobic cocci (Higashi et al). It appears that the significant incidence of *P micra* and *F nucleatum* in cases of AAA could be linked to their proteolytic capabilities (Siqueira and Rôças 2007).

Iron-acquisition genes have been shown to be enhanced in the subgingival plaque of individuals with advanced periodontitis and deep periodontal pockets, according to metagenomic research findings (Dabdoub et al). In a recent report (Ordinola-Zapata et al 2024), iron-acquisition genes (ABC transporters) were among the top 25 genes present in endodontic infections. Hemin is necessary for the growth of several anaerobic oral bacteria, including *P gingivalis*. The pathogenicity of bacteria has been linked to their capacity to collect iron ($Fe^{3+}$) from human tissues (Neilands, Akhtar and Turner). The

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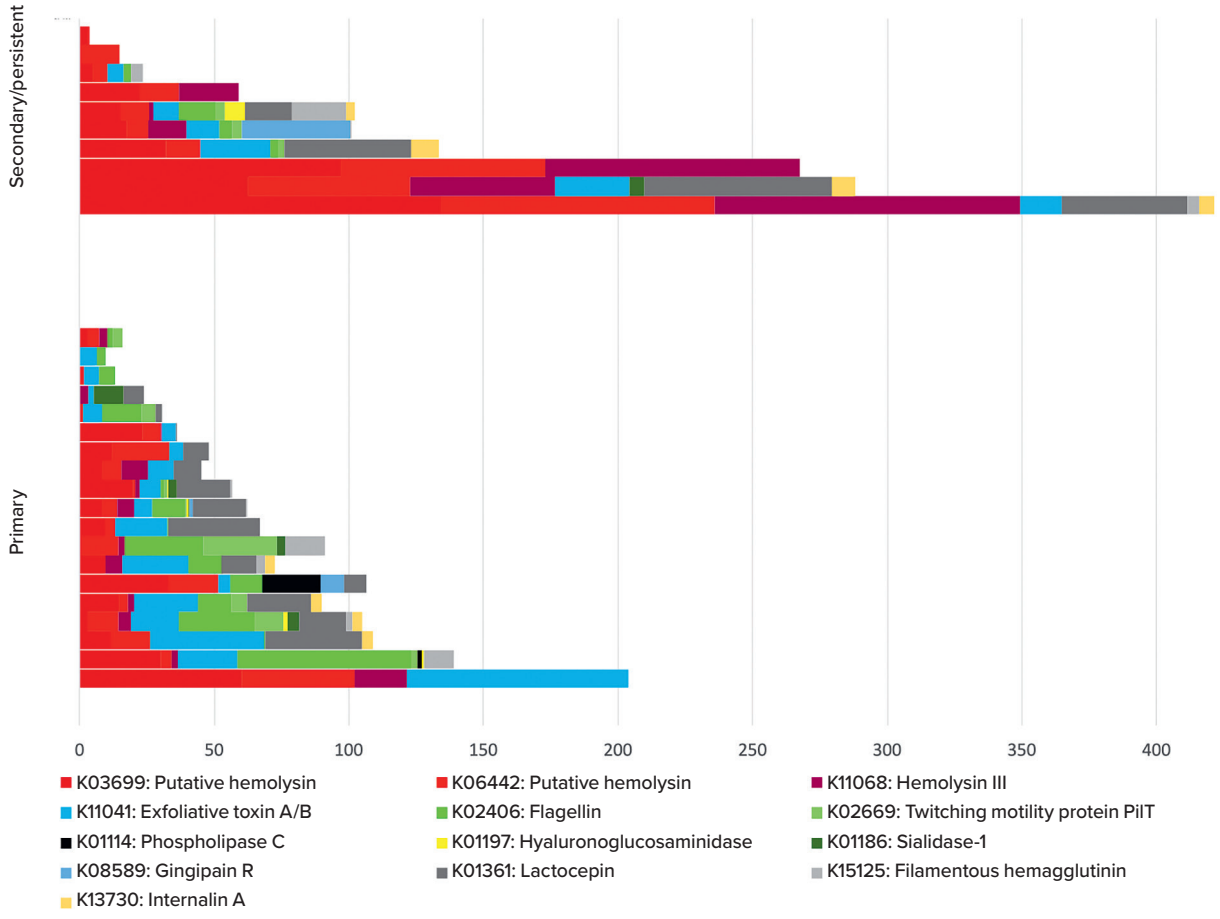
microbial diversity detected in the root canal space may be influenced by the presence of hemin-loaded proteins in the necrotic tooth pulp and periapical tissue fluids (Sundqvist 1994, Ordinola-Zapata et al 2024). In addition, several virulence factors, such as the production of outer membrane proteins, adhesins, pore-forming toxins, and hemolysins, are utilized by bacteria in the root canal to acquire iron. Among these elements, putative hemolysins are the most abundant in endodontic infections (Ordinola-Zapata et al 2024). Hemolysins generate pores in the phospholipid bilayers of eukaryotic cells (Popoff). Multiple Actinobacteria genera can contribute to the existence of putative hemolysins and hemolysin III in the root canal space, including *Olsenella*, *Actinomyces*, and *Pseudopropionibacterium (Arachnia)*. Pore-forming toxin genes can also be found in Firmicutes spp including *Streptococcus anginosus* and *Eubacterium infirmum* (Ordinola-Zapata et al 2024). It is crucial to highlight that oral bacteria such as *Porphyromonas*, *Prevotella*, and *Tannerella* spp (Bacteroidetes) may benefit from bacteria with hemolytic characteristics because their nutrient requirements are more complex, requiring not only iron but also protoporphyrin IX, a critical component of hemin (Leung and Folk, Olczak et al, Rangarajan et al). Other genes encoding toxins that degrade the extracellular matrix or cellular membranes found in the root canal include exfoliative toxin, phospholipase C, hyaluronoglucosaminidase, and sialidase (Fig 6-16) (Ordinola-Zapata et al 2024).

Proteases, fimbriae, capsular carbohydrates, and lipopolysaccharide (LPS) are essential for the growth and survival of bacteria (Casadevall and Pirofski, Steimle et al, Dahlen et al) but do not necessarily contribute to pathogenicity. For this reason, they do not fulfill the classic criteria of virulence factors. Although putative periodontal pathogens are members of the red (*Porphyromonas*, *Treponema*, *Tannerella*) and orange (*Parvimonas*, *Prevotella*, *Fusobacterium*) complexes (Socransky et al), which harbor genes encoding for proteolytic enzymes, proteolytic activity responsible for periodontal tissue destruction seems to be of host origin (Golub et al). Asaccharolytic




▲ **FIG 6-16** Individual genes encoding the most abundant toxins and virulence factors. Genes encoding hemolysins are abundant in both types of endodontic infections. Data expressed in counts per million (CPM).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microorganisms depend on the uptake of small peptides for energy supply (Grenier and Vu) and possibly produce these enzymes to degrade connective tissue and use it as a source of nutrients. The metabolism of proteins by these bacteria produces toxic sulfur products including $H_2S$ via methionine and cysteine metabolism (Foo et al), which can damage host tissues. The gene encoding the putative protease prtC U32 is frequently present in the microbiome of individuals with severe periodontitis and radicular caries (Wang et al, Damé-Teixeira et al). This protease reduces type 1 collagen, a significant component of periodontal ligament and bone. The gene prtC was also the first peptidase identified in *P gingivalis* (Kato et al). In a recent study (Ordinola-Zapata et al 2024), this gene was found in numerous anaerobic species including *Acidaminococcus intestini*, *Acidaminococcus intestini* CAG_325, *Bifidobacterium dentium*, *E infirmum*, *Desulfobulbus oralis*, *Dialister pneumosintes*, *Filifactor alocis*, *Mogibacterium diversum*, *Prevotella enoeca*, *S sputigena*, *Streptococcus oralis*, *S sanguinis*, *T denticola*, and *T forsythia*.

It is also known that bacterial proteases produced by *P gingivalis* (lysine and arginine gingipain) have evolved to use antibodies such as immunoglobulin G (IgG) as a source of energy (Vincents et al). Although genes encoding arginine gingipain (Rgp) are neither prevalent nor abundant in the root canal system, the gene prtP encoding lactocepin, a cysteine proteinase, has been reported to be present in *P micra*. Lactocepin causes degradation of inflammatory cytokines, promoting an anti-inflammatory protective effect in other microbiome contexts (von Schillde et al). *P micra* can also increase the production of gingipains from *P gingivalis*, which may increase the proteolytic activity of this pathogen (Neilands et al).

As previously mentioned, bacterial invasion of periodontal cells is a common finding in periodontal disease (Tribble and Lamont). In chronic periodontitis, specific oral bacteria can survive within eukaryotic cells and damage the attachment apparatus (Rudney et al, Graves et al). Residence within host cells allows bacteria to obtain nutrients, avoiding clearance by immune mechanisms (Tribble and Lamont). Shotgun sequencing analysis (Ordinola-Zapata et al 2024) revealed the presence of the gene encoding the protein internalin A in a common endodontic pathogen, *P micra*. As previously mentioned, internalin A is commonly used by *Listeria monocytogenes* to adhere and invade nonphagocytic cells.

Motility and adhesion are common characteristics found in pathogenic organisms that promote host invasion. Lipid A and flagellin are powerful MAMPs that engage Toll-like receptors 4 and 5, respectively, initiating MyD88-dependent and -independent release of proinflammatory cytokines (Graves et al, Kawasaki and Kawai). To date, the characterization of MAMPs in the root canal space has been focused on the presence of endotoxins or lipoteichoic acid. Gomes et al (2012) found that endotoxin concentrations were significantly higher in primary compared to secondary endodontic infections. In a recent WGS study (Ordinola-Zapata et al 2024), genes encoding lipid A were similarly abundant in both types of endodontic infections. However, it is important to recognize that the biologic significance of lipid A varies among bacterial species (Dixon and Darveau). Calcium hydroxide has been the subject of several research projects that have proved its potential to inhibit the LPS molecule (Safavi and Nichols 1993, Safavi and Nichols 1994). When LPS was mixed with calcium hydroxide and then deposited inside the root canals of dog teeth, Nelson-Filho et al observed that LPS did not elicit periapical inflammatory reactions. On the other hand, when the canals were injected with endotoxin, it resulted in a significant inflammatory response.

Multiple chemosensory pathways exist in different bacterial species, and these pathways may be expressed in response to various environmental signals. Genes encoding flagellin (a powerful immune activator) have been predominantly found in endodontic infections, mostly in *Selenomonas*, *Treponema*, *Campylobacter*, and *Oribacterium* spp (Ordinola-Zapata et al 2024). Flagella enable bacteria to penetrate cell membranes (including epithelial barriers), migrate to favorable niches, and escape from the intracellular environment (Casadevall and Pirofski). Flagella-independent motility, such as gliding and twitching, is associated with the type IV pilus in gram-negative bacteria (Melville and Craig). Bacterial adhesion to fibroblasts, hemagglutination, motility, DNA uptake, protein synthesis, and biofilm formation are just some of the processes regulated by the pilus system (Melville and Craig). Type IV (T4P) pilus (pilD, cpaF) assembly protein genes have been found in *D pneumosintes*, *D oralis*, *F alocis*, *Acidaminococcus intestini*, *P micra*, *O uli*, and *P propionicum* (Ordinola-Zapata et al 2024). Melville and Craig revealed that T4P is prevalent among gram-positive Clostridiales. Moreover, fimbrial proteins can bind with pathogen-recognition receptors on host cells, provoking the secretion of interleukin (IL)-6, IL-1β, and IL-1α from different cells (Henderson et al).

## Other microorganisms: Fungi and viruses

The vast cellular diversity of oral-dental tissues is extremely advantageous for viruses, which typically demonstrate distinct tropism for particular cell types (Thakkar et al). Surface molecules of many types (including proteins and sugar compounds) provide a chance to bind and get access to the host machinery for replication, dissemination, and persistence (Thakkar et al). Gingivitis, periodontitis, caries, and xerostomia are examples of common

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oral diseases that may allow greater access to otherwise inaccessible tissues or cells. However, while a significant number of DNA- and RNA-based viruses have been identified in the oral cavity, only a few can cause symptomatology or are typically found in damaged oral tissues, which suggests their pathogenic role (Sabeti et al 2003a, Sabeti et al 2003b, Sabeti et al 2003c, Slots, Naqvi et al 2018, Naqvi et al 2021). For example, co-infection of herpesvirus and periodontopathic bacteria may be a key pathogenic feature of juvenile periodontitis (Slots and Rams).

Viruses, primarily herpesviruses, have already been discovered in the root canal system, in addition to bacteria, (Slots et al 2003, Slots, Sabeti et al 2003a, Sabeti et al 2003b, Sabeti et al 2003c, Sabeti et al 2012, Saboia-Dantas et al, Chen et al 2009, Li et al 2009, Ferreira et al 2011a, Ferreira et al 2011b), fungi, yeasts, and archaea (Molander et al, Sundqvist et al 1998, Pinheiro et al, Siqueira 2002, Siqueira and Rôças 2004, Vianna et al, Vickerman et al, Ozok et al, Brzezińska-Błaszczyk et al). Fungi are a minor component of the oral microbiome. *C albicans* is the most common fungus species found in the oral cavity of both healthy and medically compromised individuals. It has been estimated that the frequency of *Candida* spp in primary endodontic infections is minimal (Alberti et al). Persoon et al, on the other hand, found that the frequency of fungus in primary endodontic infections associated with clinical symptoms was 53%.

While primary endodontic infections are often caused by bacteria and fungi, eukaryotes have also been identified (Koller et al). *Entamoeba gingivalis* was found to be present in the root canal microbiota in 44% of patients. The presence of these amoebas in endodontic infections could cause microbiota changes, increasing the virulence of the endodontic infection (Koller et al). Primary endodontic infections have also been linked to the Archaea domain of bacteria; the most common example is *Methanobrevibacter oralis* (Vianna et al, Brzezińska-Błaszczyk et al, Girija et al). It has been estimated that it constitutes 2.5% of the entire prokaryote community in the root canal (Vianna et al).

## Biofilms in endodontic research: Methodologic considerations

In the presence of host immunologic defenses and/or antibiotics, efficient regulatory processes are exerted among biofilm cells (Bridier and Briandet). These regulatory systems in exposed biofilm bacteria have been the subject of extensive research in the field of general medicine. The versatility of biofilm bacteria to adapt their physiology to environmental stressors has been shown to be of vital importance (Stewart, Salisbury et al, Stewart and Bjarnsholt).

In endodontics, clinical research has indicated that root canal biofilms are the main cause of primary and therapy-resistant infections (Molander et al, Sundqvist et al 1998). The natural recalcitrance of root canal biofilm communities has mainly been associated with refractory endodontic infections. In these infections, biofilm bacteria have the advantage of interacting with different species, communicating by quorum sensing, and auto-expressing resistant phenotypes. The development of resistant phenotypes is of particular interest for root canal biofilms, because these phenotypes can adapt to harsh environmental conditions such as those provoked by antimicrobials (Chavez de Paz et al 2010). Other phenotypes of interest in root canal biofilms include those with minimal to no metabolic activity. By downregulating their metabolic activity, biofilm cells can withstand long periods without consuming energy, thus lasting for very long periods of time in passive modes (Chavez de Paz et al 2008, Chavez de Paz et al 2012). The benefit of this long-term survival in a null-energy consumption mode is still unclear. However, this characteristic could explain how "silent" inflammatory lesions in periapical tissues can persist for years after root canal treatment.

Much of the knowledge regarding antimicrobial resistance of root canal bacteria in endodontics comes from studies employing single-species biofilm models—ie, biofilm developing on extracted teeth or on hydroxyapatite or plastic surfaces (Zhang et al 2009, Wang, Du et al). Ex vivo research on biofilm resistance to antimicrobials must be regarded independently; hence, generalization and definitive interpretations of such studies are not possible because the results obtained depend on the model employed (Chavez de Paz 2012, Busanello et al, Ordinola-Zapata et al 2022).

Models used in endodontics to probe antimicrobial activity involve the mechanical removal of biofilm cells, their growth on cultures, and the counting of colony-forming units (Noiri et al, Kayaoglu et al). In situ analysis of biofilms measured by fluorescent viability and confocal microscopy has also been used (Zaura-Arite et al). Some fluorescent probes detect the permeability of cell membranes as a sign of cell viability; with this method, the penetration of large fluorophores such as propidium iodide (live/dead system) into the damaged cells turns them red. As a counter stain, all cells are colored green by the general DNA-binding fluorophore SYTO 9, which effectively penetrates all cell membranes (Hu et al, Li et al 2018). See Figs 6-5 and 6-11. Other elements such as surface preconditioning have also been found to play a role in variations of biofilm structure and the distribution of viable cells with active metabolism upon the application of antimicrobial agents (Chavez de Paz et al 2010).


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## Conclusion

The development of new molecular and bioinformatics approaches is causing substantial transformation in the field of endodontic microbiology. Understanding the taxonomic organization of the microbial community is no longer sufficient, as different species or bacterial strains can show distinct pathogenic characteristics. Integrating diverse data types from various "omics" branches, such as proteomics, metabolomics, metagenomics, and transcriptomics, is crucial for achieving a comprehensive understanding of the composition and function of oral microbial communities on a global scale (Knight et al 2018). These advancements will drive applied research efforts in discovering genes, enzymes, virulence factors, and antimicrobial-resistance patterns within the endodontic microbiome. Nevertheless, it is important to acknowledge that the balance between microbial aggression and the host immune response ultimately determines the establishment of the health status. The immune status of the host should always be considered in the context of endodontic microbiology.

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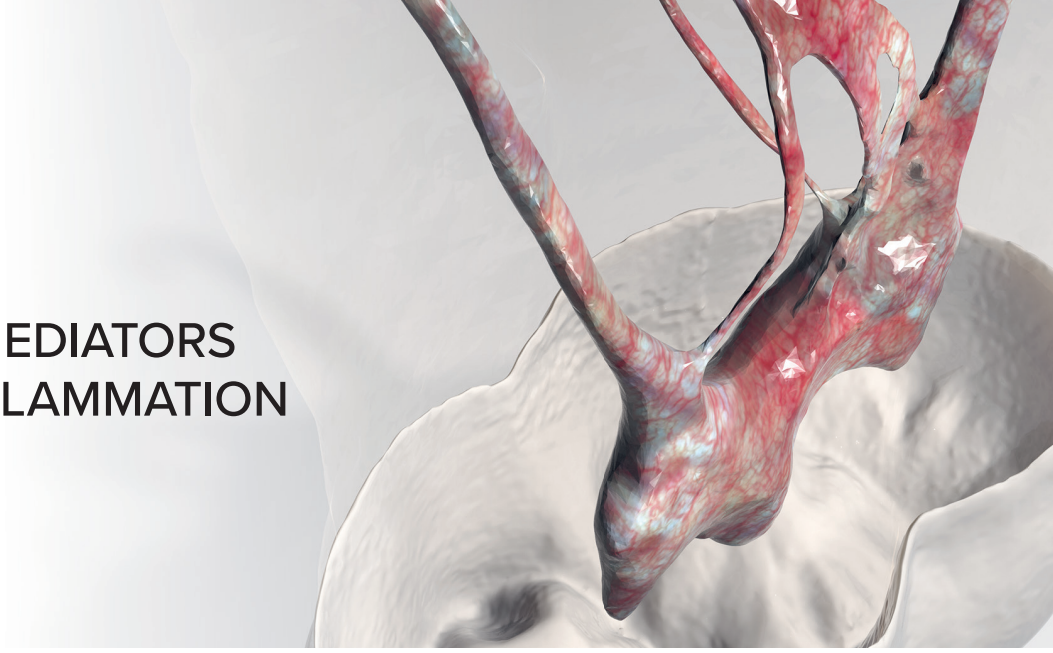

# 7

# MOLECULAR MEDIATORS OF PULPAL INFLAMMATION

Ashraf F. Fouad

Pulpitis shares many features with inflammatory changes in several other connective tissues. These include the expression of a multitude of host mediators of inflammation, as evidenced by their identification in the pulp at the protein and/or gene expression levels. Recently, it has become apparent that within the known pool of inflammatory mediators, gene polymorphism may lead to variations among individuals in the degree to which a particular gene is expressed. In addition, there are also epigenetic variations that lead to unique expression of these genes in different individuals.

The principal objective of expressed mediators is to combat the irritating factors and minimize their harmful effects. However, in the process of mounting the innate and adaptive inflammatory responses, the host factors may contribute to injury of the pulp and initiate the ultimate degeneration of this tissue. Unlike the situation in other connective tissues, the dental pulp has unique anatomical and vascular constraints that limit its recuperative potential. These constraints, which become more exaggerated with advancing age, tend to intensify the injury that results from external irritation and the harmful side effects of host inflammatory mediators. This chapter describes the data available on the contribution of molecular mediators to the inflammatory process and discusses clinical factors important for diagnosing and managing pulpal inflammation. The inflammatory response is described as it progresses from vascular changes to the attraction and migration of inflammatory cells to the site of inflammation and finally to the actual processes that take place in situ.

A large amount of data has been published in recent years about the structural composition of the dental pulp and its functional responses to external irritation. It is important to recognize that many of the functions of inflammatory and structural cells in pulpal inflammation not only overlap among the different cells but also are accomplished by molecular mediators produced by these cells. The gold standard for data generation on the inflammatory response in the dental pulp is to study these responses in humans following clinically relevant external irritation, a condition that is frequently difficult—if not impossible—to achieve. Therefore, much of the available in vitro and animal model data must be interpreted with caution and awaits confirmation by clinical studies.

## Molecular Mediators of Inflammation in Odontoblasts and Fibroblasts

It is well known that several structural cells (eg, odontoblasts, fibroblasts, osteoblasts, epithelial cells, and endothelial cells) can produce inflammatory mediators, such as cytokines or defensins, or express adhesion molecules or pattern-recognition receptors, thereby contributing to the immune response. With a function as critical as dentin formation, odontoblasts are clearly among the most important cells in the dental pulp. While odontoblasts are typically thought of as terminal protein matrix and mineralization-mediating cells, they have been shown to participate in the expression of several inflammatory mediators. Given their location as the first cells encountered when caries and sulcular bacteria invade dentin, they clearly occupy a strategic location for the recognition and mediation of various host defense responses. Therefore, odontoblasts should be considered members of the defense response cells in that they participate in the sensing of pathogens by their toll-like receptors (TLRs), produce cytokines upon cell stimulation with microbial byproducts, and induce dendritic cell migration (Fouad and Huang). Odontoblasts express TLR2 and TLR4, which detect gram-positive and gram-negative

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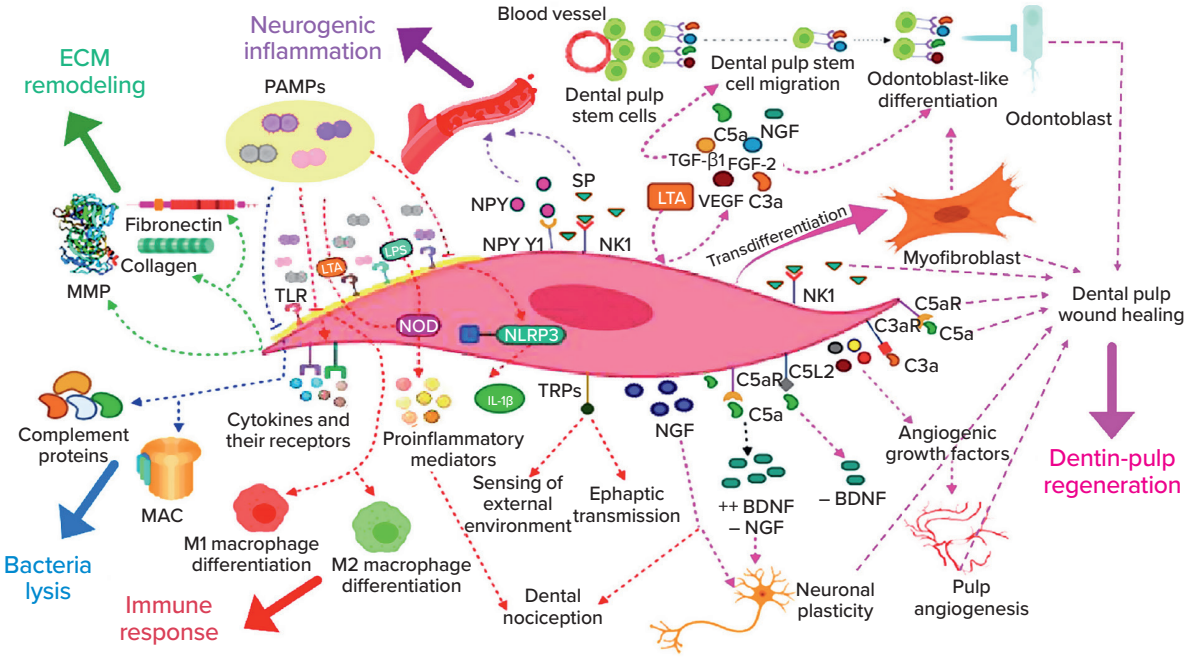
bacteria, respectively (Botero et al, Keller et al 2010, Farges et al 2015). In response to bacterial irritation, they express proinflammatory cytokines/chemokines like interleukin 1 (IL-1), tumor necrosis factor α (TNF-α), IL-8 (CXCL8), IL-6, and anti-inflammatory cytokine IL-10 (Levin et al, Farges et al 2011, Horst et al) and the antimicrobial human β-defensins (Dommisch et al 2007, Horst et al). Upon cell death, odontoblast-like cells have been shown to induce nucleotide-binding oligomerization domain (NOD)-like receptor protein 3 (NLRP3) inflammasome-dependent inflammation, which may mediate regeneration or repair in the pulp (Al Natour et al 2021a). It is also known that inflammatory mediators may mediate odontoblastic differentiation, and this was shown to be influenced by epigenetic factors like microRNA-20a (Zhang and Yuan). These findings demonstrate that odontoblasts play an important role in the production of and response to molecular inflammatory mediators.

Fibroblasts are the most abundant cell type in the pulp. They have also been shown to play a critical role in immunomodulation and defense, in addition to development, matrix synthesis, and regeneration of the dental pulp. Fibroblasts play a key role in *(1)* producing all complement system proteins as well as several proinflammatory cytokines and chemokines; *(2)* expressing pattern-recognition receptors, neuropeptides, and their receptors; *(3)* and secreting angiogenic growth factors as well as neurotrophic proteins (Alvarez-Vasquez and Castaneda-Alvarado) (Fig 7-1).




▲ **FIG 7-1** A schematic representation of the leading roles of dental pulp fibroblasts (DPFs) in pulp pathophysiology. DPFs participate in extracellular matrix (ECM) remodeling through the synthesis of collagen and fibronectin and its degradation by matrix metalloproteinases (MMPs). In response to bacterial stimulation, DPFs express all complement proteins, including the membrane attack complex (MAC), allowing the lysis of cariogenic bacteria. Furthermore, these cells have the ability to secrete and respond to various cytokines. DPFs also express the NLRP3/caspase-1 inflammasome, generating the release of IL-1β, the most potent proinflammatory cytokine. They express toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD) receptors and thus recognize pathogen-associated molecular patterns (PAMPs) and regulate the expression of various proinflammatory mediators through the recognition of PAMPs like lipoteichoic acid (LTA) and lipopolysaccharide (LPS). In response to inflammatory mediators, DPFs secrete substance P (SP) and express receptors for neuropeptides such as neurokinin-1 (NK1) receptor and neuropeptide Y Y1 (NPY Y1) receptor, thus participating in neurogenic inflammation and dental pulp wound healing and amplifying the pulp immune response. DPFs contribute to dental nociception by secretion of nerve growth factor (NGF), which sensitizes afferent nerve fibers and expresses transient receptor potential (TRP) channels that are sensors of the external environment. TRP channels could allow DPFs to perform ephaptic transmission, although this is yet to be elucidated. DPFs interact with macrophages and modulate their differentiation into M1 (proinflammatory) macrophages to control infection and M2 (anti-inflammatory) macrophages to start pulp healing, demonstrating the active participation of DPFs in local immune response and inflammation. DPFs also actively participate in dentin-pulp regeneration through the secretion of growth factors such as transforming growth factor β1 (TGF-β1), basic fibroblast growth factor (FGF-2), vascular endothelial growth factor (VEGF), complement proteins (C3a and C5a), and NGF, allowing dental pulp stem migration and odontoblast-like differentiation. They also secrete angiogenic growth factors for pulp angiogenesis. DPFs secrete the brain-derived neurotrophic factor (BDNF) when C5a binds to its C5aR receptor, decreasing the expression of NGF (which is increased in inflamed pulp tissue); BDNF decreases when C5a binds to the C5L2 receptor. Both NGF and BDNF are essential for neuronal plasticity. Finally, DPFs participate in dental pulp wound healing and dentin-pulp regeneration by transdifferentiating into myofibroblasts; these latter cells facilitate the reorganization of the ECM in injured pulp and can differentiate into odontoblast-like cells, with the capacity to synthesize reparative dentin. (Reprinted with permission from Alvarez-Vasquez and Castaneda-Alvarado.)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## Modulation of Neurogenic Inflammation

In the initial phases of pulpal inflammation, there is vasodilation and increased blood flow. This serves the function of increased perfusion of the pulp, bringing needed host inflammatory factors into the area of irritation. Despite its small size, the dental pulp responds to advancing irritation in a compartmentalized manner rather than as an entire organ. Areas of the pulp closest to the irritation seem to be affected the most and undergo more severe inflammatory manifestations with resultant vascular responses, while the surrounding and distant sections may have milder inflammation or noninflamed tissue (Ricucci et al). As the inflammatory reaction in the pulp progresses, stasis in pulp vessels eventually ensues. This is related to the extravasation of proteins and cells into the interstitial tissue, as well as the nonyielding dentin environment that restricts tissue edema. The vascular responses of the pulp are mediated by several vasoactive amines, including histamine, serotonin, endothelin, and neuropeptides.

### Histamine

Histamine is prevalent in the connective tissue around blood vessels and in mast cells, basophils, and platelets. It is present in cell granules that are released by cell degranulation in response to a variety of stimuli (Kumar et al):

- Physical trauma, cold, or heat
- Immune reactions involving binding of antibodies (immunoglobulin E [IgE]) to mast cells
- Complement components called *anaphylatoxins* (C3a and C5a)
- Histamine-releasing proteins derived from leukocytes
- Neuropeptides (eg, substance P)
- Cytokines such as IL-1 and IL-8

Histamine is a potent vasodilator and a mediator of vascular permeability. It acts on the microcirculation via four types of G-protein-coupled receptors: $H_1$, $H_2$, $H_3$, and $H_4$ receptors. The $H_4$ receptor is involved in chemotaxis and inflammatory mediator release by eosinophils, mast cells, monocytes, dendritic cells, and T cells (Kumar et al).

The application of histamine to the pulp in vivo resulted in vasodilation (Edwall et al) and gradual decrease in pulpal blood flow (PBF) (Kim and Dörscher-Kim) due to vascular leakage and an increase in tissue pressure in a low-compliance environment. Histamine application to cavity preparations in human dentin resulted in dull throbbing pain and was occasionally associated with sharp, shooting pain (Ahlquist and Franzen). Because histamine has little stimulation of A- or C-fibers in the dental pulp, the pulp tissue must be sensitized by other inflammatory mediators such as prostaglandins for histamine to evoke a painful response in either type of pulp nociceptive fiber (Ahlquist and Franzen).

### Serotonin

Serotonin (5-hydroxytryptamine or 5-HT) is another vasoactive mediator that generally causes vasoconstriction. Serotonin is also an important signaling molecule for the development and maintenance of mineralized tissues, including dentin (Baudry et al). It is present in serotonergic nerve terminals, endothelial cells, and platelets. Release of serotonin (and histamine) from platelets occurs after they come into contact with collagen, thrombin, adenosine diphosphate (ADP), and antigen-antibody complexes. Serotonin and the enzyme monoamine oxidase, which catalyzes the oxidative deamination of 5-HT, have been localized in the linings of blood vessels in normal human dental pulp (Inoue et al) and have been shown to increase the biosynthesis of endogenous prostaglandin $E_2$ ($PGE_2$) (Hirafuji et al) and prostacyclin ($PGI_2$) (Hirafuji and Ogura 1987) in the rat dental pulp. Serotonin also caused significant increase in PBF in dog canines when injected in the maxillary artery or applied locally into Class V cavities (Liu et al 1990) and was shown to sensitize pulpal nerve fibers to various hydrodynamic stimuli (Ngassapa et al), indicating that it is capable of reducing the pain threshold in pulpal inflammation. One study showed that 5-HT induced a significant increase in human capsaicin-evoked calcitonin gene-related peptide (CGRP), but only in females (Loyd et al).

### Endothelin

Endothelin is a potent, trypsin-sensitive vasoconstrictor and a multifunctional peptide originally identified within endothelial cells and subsequently in some epithelial cells, neurons, and neuroendocrine cells (Casasco et al 1991). It was shown to be present in normal and inflamed human pulp (Casasco et al 1992). The genes for three isoforms of endothelin (ET-1, ET-2, and ET-3) have been cloned. Two types of responses to endothelin have been described, which correspond to two receptors: endothelin receptor A (ET-A), which is specific for ET-1, and endothelin receptor B (ET-B), which is specific for all isoforms (Masaki et al). Endothelin has various physiologic actions in addition to vasoactivity, because receptors are found in the brain, adrenal glands, and lung; in addition, knockout studies show significant structural and developmental abnormalities (Neuhaus and Byers). ET-A and ET-B as well as ET-1 have also been detected in developing rat dental tissues, including odontoblasts, ameloblasts, and dental papillae (Neuhaus and Byers).

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**BOX 7-1 Modulation of inflammation by nitric oxide**

| Proinflammatory properties | Anti-inflammatory properties |
|---|---|
| • Promotes vasodilation and vascular leakiness | • Inhibits leukocyte adhesion to endothelium |
| • Promotes hypotension or vascular collapse in sepsis | • Inhibits P-selectin expression by platelets, endothelium |
| • Cytotoxic | • Inhibits microvascular thrombosis |
| • Activates cyclooxygenase (depends on the dose) | • Inhibits lymphocyte proliferation |
| • Reacts with oxygen to form toxic peroxynitrite | • Inhibits mast cell degranulation |
| • Inhibitors of nitric oxide (NO) synthesis ameliorate experimental models of arthritis | • Inhibits oxidant production by phagocytes |
| • Simulates TNF-α production by synoviocytes | • Inhibits cyclooxygenase (depends on the dose) |

Based on Clancy et al.

Intra-arterial delivery of ET-1 in rats produced a dose-dependent reduction of PBF exceeding that caused by epinephrine and norepinephrine (Yu et al 2002). Similar effects were achieved when ET-1 was applied extraluminally to porcine pulp preparations (Yu et al 2004). However, blocking ET-A with an antagonist did not change basal PBF (Berggreen and Heyeraas 2003). ET-1 promotes odontogenic differentiation of dental pulp stem cells (DPSCs) (Liu et al 2018).

## Neuropeptides

Many neuropeptides have been identified in the dental pulp, including substance P (SP), CGRP, neurokinin A (NKA), neuropeptide K, neuropeptide Y (NPY), somatostatin, and vasoactive intestinal peptide (VIP) (Caviedes-Bucheli et al 2021). These neuropeptides reside almost exclusively under normal conditions within the nerve endings of afferent nerves and are usually (but not exclusively) located around pulpal blood vessels. Of the 20% of pulpal blood vessels that have positive peptidergic innervations, 92% expressed CGRP, 87% expressed SP, 80% expressed NPY, and 15% expressed VIP (Rodd and Boissonade 2003). Tissue levels of SP immunoreactivity in the dental pulp were found to be the highest in the body outside the central nervous system and were higher in mature than immature cat pulp (Brodin et al). Dental pulp fibroblasts express SP, demonstrating their role in neurogenic inflammation in pulpal disease (Killough et al). Denervation experiments indicate that, in the pulp, SP-, NKA-, and CGRP-containing nerve fibers originate from the trigeminal ganglion and NPY-containing nerve fibers come from the superior cervical ganglion. The origin of VIP-containing fibers may be the parasympathetic ganglia because VIP has been associated with acetylcholine. The origin of pulpal neuropeptides, their receptors, and their functions are summarized in Box 7-1 and Table 7-1.

Increased production of neuropeptides plays an important role in initiating and propagating pulpal inflammation. During inflammation induced by caries, cavity preparation, or the application of orthodontic forces, sprouting of pulpal nerve fibers was shown to be associated with increased expression of neuropeptides such as SP or CGRP closely surrounding the areas of irritation (Kimberly and Byers, Byers, Caviedes-Bucheli et al 2008). Studies have also shown that the amounts of NPY or VIP in the pulp of mild to moderately carious teeth were significantly more than those present in noncarious or grossly carious teeth (El Karim et al 2006a, El Karim et al 2006b). Conversely, a higher percentage of neuronal areas that were positively stained for SP was detected in grossly carious lesions and in painful lesions (Rodd and Boissonade 2000). These findings were consistent at all levels of the coronal pulp, subodontoblastic nerve plexus, and midcoronal pulp.

Teeth diagnosed with painful irreversible pulpitis contained increased levels of SP or its receptor, CGRP or its receptor, NKA, and NPY (Caviedes-Bucheli et al 2008). Immunoreactive CGRP (iCGRP) has often been used as a marker for assessing neurogenic inflammation. A decrease in pH and increase in temperature significantly increased the effect of capsaicin on CGRP release from pulp nociceptors (Goodis et al 2006). The results suggest that neuropeptide concentration, local inflammation, and body heat may influence the intensity of pulpal pain. Moreover, levels of SP and NKA increased in the gingival crevicular fluid of teeth with painful irreversible pulpitis, and the levels returned to normal 1 week after pulpectomy (Awawdeh et al). However, other data suggests that neuropeptides like SP and CGRP are significantly increased in asymptomatic forms of pulpitis induced by occlusal trauma, intrusion, or other forms of orthodontic movement (Caviedes-Bucheli et al 2011, Chavarria-Bolanos et al, Caviedes-Bucheli et al 2021).


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**TABLE 7-1** A summary of pulpal neuropeptides, receptors, and their functions

| Neuropeptide | Origin | Localization | Stimulus for release | Cell receptor | Cell expressing the receptor | Biologic effect |
|---|---|---|---|---|---|---|
| Substance P | Trigeminal ganglion | C- and Aδ-fibers | • Thermal<br>• Mechanical<br>• Chemical<br>• Electrical<br>• Caries<br>• Capsaicin<br>• Inflammatory mediators<br>• Bradykinin<br>• Prostaglandins | NK1 | • Mast cells<br>• Macrophages<br>• Mesenchymal cells<br>• Odontoblasts<br>• Fibroblasts<br>• Lymphocyte T<br>• Lymphocyte B | • Vasodilation<br>• Plasma extravasation<br>• Immune system stimulator<br>• Chemotaxis<br>• Enhances macrophage activity<br>• Hard tissue formation<br>• Tissue reparation<br>• Mitogen for lymphocyte T |
| Neurokinin A | Trigeminal ganglion | C- and Aδ-fibers | • Thermal<br>• Mechanical<br>• Chemical<br>• Electrical<br>• Caries<br>• Capsaicin<br>• Inflammatory mediators<br>• Bradykinin<br>• Prostaglandins | NK2 | • Mast cells<br>• Macrophages<br>• Mesenchymal cells<br>• Lymphocyte T<br>• Lymphocyte B | • Vasodilation<br>• Plasma extravasation<br>• Chemotaxis<br>• Pain |
| Calcitonin gene-related peptide | Trigeminal ganglion | C- and Aδ-fibers | • Thermal<br>• Mechanical<br>• Chemical<br>• Electrical<br>• Caries<br>• Capsaicin<br>• Inflammatory mediators<br>• Bradykinin<br>• Prostaglandins | CGRP1, CGRP2 | • Mast cells<br>• Macrophages<br>• Mesenchymal cells<br>• Fibroblasts<br>• Ameloblasts<br>• Lymphocyte T<br>• Lymphocyte B | • Vasodilation<br>• Plasma extravasation<br>• Chemotaxis<br>• Lymphocyte T suppressor<br>• Hard tissue formation<br>• Repair process<br>• Mitogen for odontoblasts<br>• Pain<br>• Resorption control |
| Neuropeptide Y | Superior cervical ganglion | Sympathetic fibers | • Stress<br>• Electrical<br>• Thermal<br>• Caries | Y receptor | • Smooth muscle cells | • Vasoconstriction<br>• Pain<br>• Repair process |
| Vasoactive intestinal peptide | Parasympathetic ganglia | Parasympathetic fibers | • Not well elucidated<br>• Cytokines<br>• Lipopolysaccharides<br>• Nitric oxide | VPAC1, VPAC2 | • Sensory neurons, mesenchymal cells<br>• Osteoblasts<br>• Osteoclasts<br>• Mast cells<br>• Macrophages<br>• Lymphocyte T<br>• Lymphocyte B | • Vasodilation<br>• Immunomodulator<br>• Regulates inflammation |

Modified with permission from Caviedes-Bucheli et al 2008.

Not all cases of irreversible pulpitis are associated with pain. This may be explained by the actions of inhibitors of neurotransmitters such as gamma-aminobutyric acid (GABA) or gastrin-releasing peptide (GRP). GABA-like and GRP-like immunoreactivity has been identified in the dental pulp (Todd et al). Mu-opioid receptors have also been identified in human dental pulp and may be involved in suppressing pain symptoms in certain cases (Jaber et al, Chavarria-Bolanos et al). The Y1 receptor for NPY has been shown to colocalize with the capsaicin receptor TRPV1 (transient receptor potential cation channel subfamily V member 1) in the trigeminal ganglion and the dental pulp. Thus, activation of the Y1 receptor by endogenous NPY results in the inhibition of capsaicin-sensitive nociceptors,

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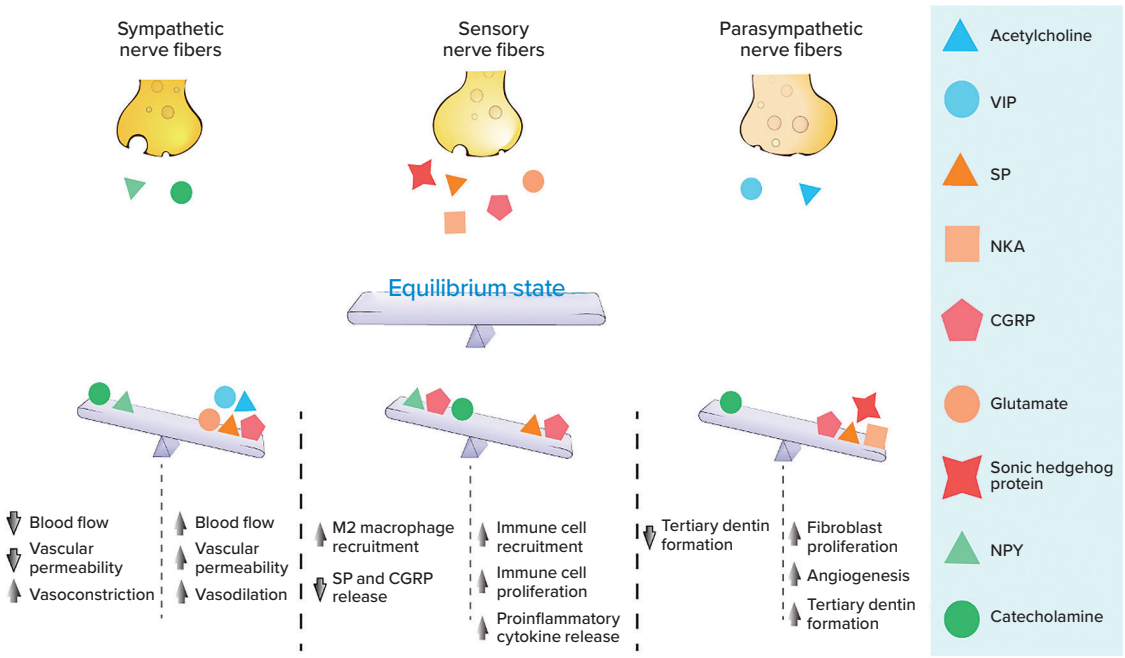


▲ **FIG 7-2** Imbalance of dental nerves in pulpitis. Pulpal sensory, sympathetic, and parasympathetic nerve fibers may regulate the vasculature and immune response in pulpitis via communication with relevant cells through molecular factors. Vasodilator function of neuropeptides and glutamate overwhelm the vasoconstrictor function of sympathetic nerves in pulpitis. Meanwhile, to some extent, the acetylcholine and VIP secreted by parasympathetic nerve fibers also lead to vasodilation and increased vascular permeability. SP promotes an immune reaction, resulting in a proinflammatory response, while neuronal signaling molecules from sympathetic nerve fibers have an immune-suppressive function. CGRP may either promote migration of immune cells or be involved in immunosuppression. Various molecules including SP, NKA, CGRP, and the Sonic hedgehog protein (Shh) released from sensory nerves participate in tissue repair activity. In contrast, catecholamine decreases tertiary dentin formation. (Reprinted with permission from Zhan et al.)

thereby modulating the intensity of the pain response (Gibbs and Hargreaves). There are several mechanisms by which neuropeptides are thought to contribute to the inflammatory process (Fig 7-2). SP, CGRP, and VIP (Gazelius et al 1987) are potent vasodilators, while NPY is a vasoconstrictor (Wakisaka and Akai). Vasodilation following infusion of SP or VIP in the pulp was associated with a brief increase in PBF, which was followed by a dramatic and prolonged reduction in PBF (Kim and Dörscher-Kim). Intra-arterial infusion of an SP antagonist reduced resting blood flow and interstitial fluid pressure in the ferret canine pulp (Berggreen and Heyeraas). SP also increased vascular permeability and plasma extravasation (Hargreaves et al). The use of β-adrenoceptors epinephrine and norepinephrine as well as albuterol (a β2-adrenoceptor agonist) inhibited CGRP release from pulpal nociceptors in ex vivo bovine pulp preparations (Bowles et al). The different stimuli that trigger neuropeptide release and their role in neurogenic inflammation are summarized in the review by Caviedes-Bucheli et al (2008).

Neuropeptides may also contribute to the inflammatory process by other mechanisms. Application of SP or CGRP to human pulp fibroblasts upregulated the production of IL-1, IL-6, and TNF-α in a dose- and time-dependent manner (Yamaguchi et al). Similar effects were shown on the production of IL-8, IL-10, cyclooxygenase (COX)-2, and monocyte chemotactic factor-1 (MCP-1) (Patel et al, Park et al 2004, Tokuda et al). These effects seem to be mediated by the interactions of nuclear factor κB (NFκB) and the receptor antagonist for its ligand (RANKL) (Tokuda et al, Kojima et al). The interactions between neuropeptides and different cell populations of the dental pulp are summarized in the review by Caviedes-Bucheli et al (2008) (Fig 7-3).

Neuropeptides also play a role in pulp regeneration and reparative odontogenesis. For example, SP was involved in DPSC proliferation and odontogenic differentiation through its binding to the neurokinin-1 receptor (NK1) (Wei et al 2023); however, CGRP inhibited DPSC viability and differentiation (Michot et al 2020). These antagonistic


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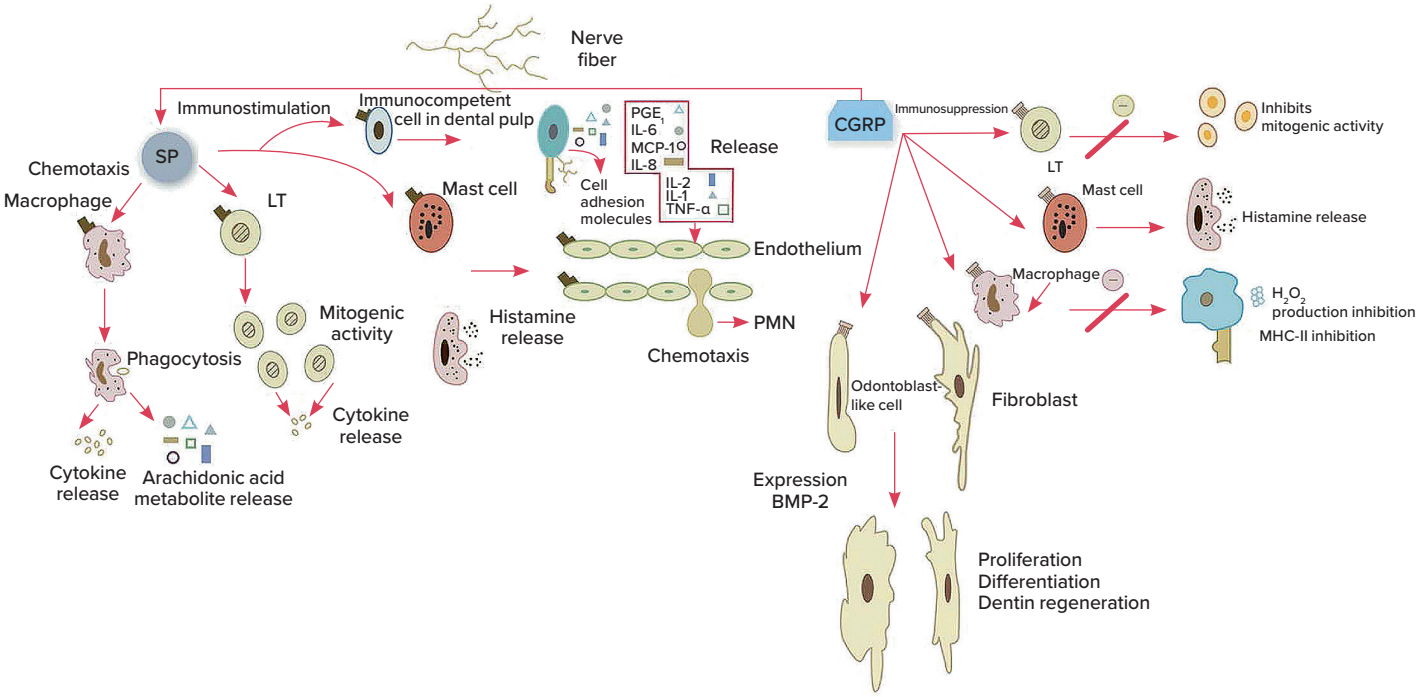


▲ **FIG 7-3** Interactions between neuropeptides and different cell populations of the dental pulp. Neuropeptides initiate and spread neurogenic inflammation. SP generates immunostimulatory effects by enhancing macrophage phagocytosis, chemotaxis, and cytokine release. In other immunocompetent cells, SP induces the release of $PGE_2$, IL-1, IL-2, IL-6, IL-8, and TNF-α. SP also regulates the expression of MCP-1, as well as the mitogenic activity and cytokine release of T lymphocytes (LT), and promotes neutrophil (PMN) chemotaxis. Both SP and CGRP induce histamine release from mast cells. CGRP inhibits mitogen-induced T-lymphocyte proliferation, blocks hydrogen peroxide ($H_2O_2$) production in macrophages, and reduces antigen presentation of class II antigen-expressing cells (MHC-II). CGRP exerts stimulatory effects on the growth of fibroblasts and odontoblast-like cells by increasing the expression of bone morphogenetic protein 2 (BMP-2), thereby inducing dentin regeneration and lowering dentin permeability. (Reprinted with permission from Caviedes-Bucheli et al 2008.)

functions may relate to actions at various degrees of pulpal inflammation and regeneration.

## Leukocyte Adhesion and Transmigration

### Adhesion molecules

One of the critical processes in inflammation is the delivery of leukocytes to the site of inflammation. As the blood flow slows down due to vessel vasodilation and increased vascular permeability, the leukocytes assume a more peripheral position in the vessel (margination). Eventually, the leukocytes roll along the endothelial wall and finally adhere to the endothelial lining (pavementing). They then insert pseudopods into gaps between endothelial cells, transmigrate through the basement membrane, and travel toward the site of inflammation following the chemotactic gradient (Fig 7-4).

Important classes of molecules that are critical for the adhesion and transmigration of leukocytes include selectins, integrins, and endothelial adhesion molecules. Selectins are the molecules that initially slow down the circulating leukocytes and cause them to roll along the endothelial lining. L-selectins are found on leukocytes, whereas P- and E-selectins are expressed by endothelial cells (Langer and Chavakis). The absence of fucosyltransferase, which is required for the synthesis of an oligosaccharide receptor for P-selectins and E-selectins, causes a condition called *leukocyte adhesion deficiency-II* (LAD-II) (Kumar et al). In the normal dental pulp, only a few vessels located in the pulp core reacted weakly with an immunohistochemical stain for E- or P-selectins. However, in the inflamed pulp, these molecules are upregulated (Fouad 2022).

Cell adhesion molecules are members of the immunoglobulin (Ig) superfamily and include intercellular

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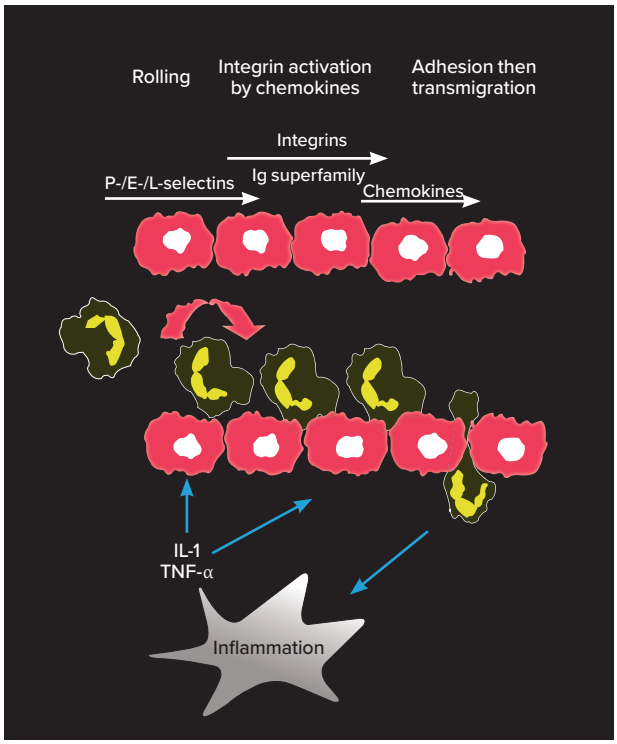


▲ **FIG 7-4** The recruitment of leukocytes to sites of inflammation depends on adhesive interactions between leukocytes and endothelial cells or endothelial cell–bound platelets. The production of cytokines in inflammatory tissues (such as IL-1 and TNF-α) leads to the expression of E- and P-selectins on the walls of endothelial cells (and L-selectins on leukocytes), which results in the rolling of the inflammatory cells. The combination of high-affinity integrins (lymphocyte function-associated antigen 1 [LFA-1], macrophage-1 antigen [Mac-1], and very late antigen-4 [VLA-4]) and immunoglobulin superfamily members (intercellular adhesion molecules [ICAMs], platelet endothelial cell adhesion molecules [PECAMs], and vascular cell adhesion molecules [VCAMs]) on the endothelial cell surface and the presence of leukocytes and chemokines in inflamed tissues results in the attachment of the inflammatory cells and transmigration to the site of inflammation, infection, or tissue injury.

adhesion molecules (ICAMs) 1 to 5, vascular cell adhesion molecule-1 (VCAM-1), junctional adhesion molecules (JAMs), platelet endothelial cell adhesion molecule-1 (PECAM-1), and endothelial cell-selective adhesion molecule (ESAM) (Langer and Chavakis). In addition, apart from their well-established role as the first cellular response in the coagulation cascade, platelets are intimately involved in inflammatory reactions, largely because of their direct crosstalk with leukocytes (see Fig 7-4) (von Hundelshausen et al). ICAM-1 is strongly induced on leukocytes and endothelial cells. Endothelial cells constitutively express ICAM-2, VCAM-1, and PECAM-1, whereas resting leukocytes bear ICAM-3 and PECAM-1 on their surfaces. ICAM-1 interacts with the integrins lymphocyte function-associated antigen 1 (LFA-1) and macrophage-1 antigen (Mac-1), ICAM-2 and ICAM-3 interact with LFA-1, and VCAM-1 interacts with very late antigen-4 (VLA-4). PECAM-1 and JAMs are engaged in cell binding, which allows the phagocyte to squeeze between or through the endothelial cells, a process called *transmigration* or *diapedesis* (Fouad and Huang, Abbas et al). After their firm adhesion, leukocytes make use of two transmigration processes to cross the endothelial barrier: the transcellular route through the endothelial cell body and the paracellular route through the endothelial junctions.

Immunohistochemical studies have localized the presence of PECAM-1, ICAM-1, and ICAM-2 on the endothelium of capillaries, arterioles, and venules in the human dental pulp. In addition, ICAM-3 was detected on peripheral blood cells and VCAM-1 was weakly recognized from the venule endothelium. P-selectin was mostly located on venule endothelium and platelets (Tasman et al 1999).

Integrins are transmembrane glycoproteins expressed on many cell types, including leukocytes and endothelial cells. Their cytoplasmic domains bind to the cytoskeleton. The integrin superfamily consists of about 30 structurally homologous proteins that promote cell-cell or cell-matrix binding. All integrins are heterodimeric cell surface proteins consisting of an α chain and a β chain that are noncovalently linked. The most notable integrins found on leukocytes are LFA-1, Mac-1, and VLA-4. The major function of integrins is to mediate stable adhesion of leukocytes to endothelial cells, T cells to antigen-presenting cells, cytolytic T cells to target cells, and connective tissue cells to extracellular matrix (ECM) proteins such as fibronectin, vitronectin, osteopontin, and collagen (Takada et al). Patients who have a defect in the biosynthesis of the β2 chain, which is shared by LFA-1 and Mac-1 integrins, develop leukocyte adhesion deficiency-I (LAD-I). Patients with LAD-I and LAD-II (discussed previously) develop immunodeficiency and recurrent bacterial infections (Kumar et al).

Human dental pulp cells were found to express α1, α3, α5, α6, αv, and β1 integrin subunits by immunoblot or immunoprecipitation techniques (Zhu et al 1998a, Zhu et al 1998b). Integrin chains α1, α2, α3, α5, α6, and β1 were also immunohistochemically demonstrated in the normal dental pulp (Tasman et al 2001). In the dental pulp, integrins are essential for adhesion of odontoblasts to each other as well as to predentin, other pulp cells, and matrix proteins. Mature odontoblasts were found to express αvβ1, αvβ3, and αvβ5 genes (Lucchini et al, Staquet et al).


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## Chemotactic factors

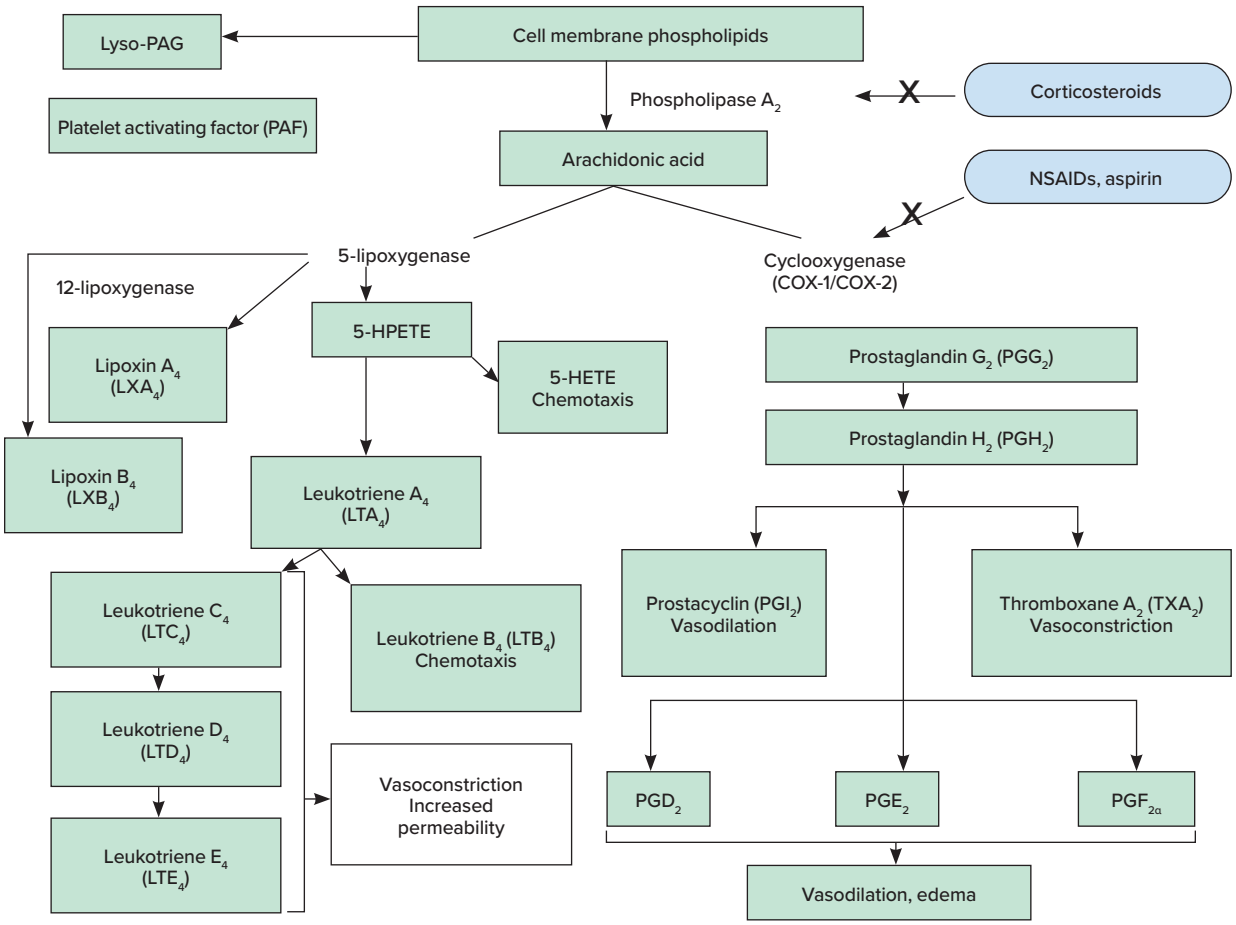
After transmigration, leukocytes are attracted to the site of inflammation along a chemical gradient set by several chemotactic factors. Bacterial products can act as chemoattractants. Studies conducted in primates by Bergenholtz et al have shown that the application of bacterial components to Class V cavities caused the attraction of neutrophils to subjacent pulp areas within hours (Warfvinge et al).

Endogenous molecules that mediate chemotaxis include complement components C3a and C5a, leukotrienes (especially leukotriene $B_4$ [$LTB_4$]), and chemokines (such as IL-8/CXCL8). (A more detailed discussion of these factors follows later in the chapter.) It has been shown that vascular endothelial growth factor (VEGF), an angiogenic growth factor that induces proliferation and migration of vascular endothelial cells, promotes chemotaxis and proliferation of human pulp cells (Matsushita et al). These effects were in part mediated by the activation of the DNA-binding proteins activator protein-1 (AP-1) and, to a lesser degree, NFκB.

# Other Mediators of the Inflammatory Response

## Arachidonic acid metabolites

When cells are activated, their membrane phospholipids are rapidly remodeled to generate biologically active lipid inflammatory mediators. Products derived from the metabolism of arachidonic acid, a 20-carbon polyunsaturated fatty acid, affect a variety of biologic processes, including inflammation and hemostasis. Arachidonic acid metabolites, also called *eicosanoids*, are synthesized by two major classes of enzymes: cyclooxygenases (prostaglandins and thromboxanes) and lipoxygenases (leukotrienes and lipoxins) (Fig 7-5). A third metabolic pathway involving cytochrome P450 epoxygenases metabolizes arachidonic acids into several epoxyeicosatrienoic acids (EETs). The EETs have also been shown to play critical roles in regulating cellular proliferation, inflammation, hemostasis, and a variety of intracellular signaling pathways.




▲ **FIG 7-5** Phospholipid metabolism and arachidonic acid pathways. HETE = hydroxyeicosatetraenoic acid; HPETE = hydroperoxyeicosatetraenoic acid; NSAIDs = nonsteroidal anti-inflammatory drugs.

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### Prostaglandins and thromboxanes

The cyclooxygenase (COX) pathway leads to the generation of prostaglandins. This process takes place in the normal pulp and is blocked by the addition of indomethacin, a prostaglandin synthetase inhibitor, and augmented by exogenous arachidonic acid or serotonin (Khanapure et al). The most important prostaglandins in inflammation are $PGE_2$, $PGD_2$, $PGF_{2\alpha}$, $PGI_2$ (prostacyclin), and thromboxane $A_2$ ($TXA_2$). It has been known for decades that prostaglandins play a critical role in the pathogenesis of pulpal disease (Torabinejad and Bakland 1980a, Torabinejad and Bakland 1980b). Induced pulpal inflammation in the rat incisor using bacterial lipopolysaccharide (LPS) resulted in a 9.3-fold increase in $PGE_2$ and 3.8-fold increase in 6-keto-$PGF_{1\alpha}$, a stable metabolite of $PGI_2$ (Okiji et al 1987). Increased levels of $PGE_2$, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$ have been demonstrated immunohistochemically in the pulp following induced inflammation (Miyauchi et al). Prostaglandins may induce the production of other inflammatory mediators or regenerative molecules as well. In the dental pulp, $PGE_2$ significantly increased the bradykinin-evoked release of iCGRP (Goodis et al 2000) and the production of hepatocyte growth factor, a factor that stimulates DNA synthesis (Ohnishi et al).

Prostaglandins are also involved in the pathogenesis of pain. Intravenous administration of nonsteroidal anti-inflammatory drugs (NSAIDs), which are known to block the COX pathway, resulted in significant inhibition of stimulated nerve activity in the feline pulp (Ahlberg). Furthermore, patients with severe pulpal pain had significantly increased pulpal levels of $PGE_2$ and $PGF_{2\alpha}$ when compared with patients with nonpainful pulpitis or normal pulp (Cohen et al). Injection of the steroid Depo-Medrol (Pfizer) via an intraosseous route to teeth with irreversible pulpitis significantly reduced $PGE_2$ after 1 day in the pulp (Isett et al). Because prostaglandins are abundant in pulpal inflammation, it has been suggested that their level could be measured to determine the degree of pulpal inflammation of patients undergoing pulpotomy procedures in primary teeth in order to predict long-term treatment outcomes (Waterhouse et al 1999, Waterhouse et al 2002).

Glucocorticoids block the breakdown of membrane phospholipids to arachidonic acid by phospholipases, whereas NSAIDs block the COX pathway. Investigations have shown the effectiveness of corticosteroids (Chance et al, Fachin and Zaki, Rogers et al) and NSAIDs (Curtis et al, Doroschak et al) on pulpal pain, particularly in apical inflammation that occurs following pulpectomy. The COX pathway is mediated by at least two different enzymes: COX-1 and COX-2. COX-1 is constitutively expressed and has beneficial homeostatic functions on the gastric mucosa and the kidneys, whereas COX-2 is an induced proinflammatory enzyme (Colville-Nash and Gilroy). Several reports have documented that neuropeptides and cytokines induce COX-2 expression in pulp cells in vitro (Chang et al 2003, Kojima et al) and in the pulp in vivo (Ribeiro-Santos et al, Jungbluth et al). Moreover, genetic variants of the COX-2 gene influence posttreatment pain in endodontic patients (Applebaum et al).

### Leukotrienes and lipoxins

The lipoxygenase pathway products are only present in inflammatory cells such as neutrophils, eosinophils, mast cells, basophils, macrophages, and monocytes. This is distinctly different from COX products, which are present in all mammalian cells except erythrocytes (ie, all nucleated cells). 5-lipoxygenase is the predominant enzyme in neutrophils. The main product, 5-hydroxyeicosatetraenoic acid (5-HETE), which is chemotactic for neutrophils, is converted into a family of compounds collectively called *leukotrienes*. $LTB_4$ is a potent chemotactic agent and activator of neutrophil functional responses, such as aggregation and adhesion of leukocytes to venule endothelium, generation of oxygen free radicals, and release of lysosomal enzymes (Khanapure et al, Kumar et al). Inflammation induced in animal dental pulps demonstrated increased production of $LTB_4$ (Okiji et al 1991, Hutchins et al) or leukotriene $C_4$ ($LTC_4$) (Lessard et al). A dual inhibitor of the COX and lipoxygenase pathways inhibited $LTB_4$ or $LTC_4$ production, whereas indomethacin had no effect (Okiji et al 1991). Minor changes in temperature increased $LTB_4$ expression in human primary pulp cells and pulp stem cells (Eberhard et al).

The results of studies on whether leukotrienes reduce pain threshold in the dental pulp have not been conclusive. $LTB_4$ and $LTC_4$ significantly reduced spontaneous and evoked nerve excitability of the cat dental pulp in one study (Gazelius et al 1984). However, $LTB_4$ increased pulpal nerve excitability under similar conditions in another study (Madison et al). More recently, it was shown that $LTB_4$ modulated the odontoblastic differentiation and mineralization by increased expression of the IBSP (integrin-binding sialoprotein) and Runx2 (runt-related transcription factor 2) genes in DPSCs (da Silva et al).

Although inflammation is the key process in host defense, the resolution of an inflammatory response is equally important to reestablish homeostasis and limit excessive tissue injury. Lipoxins are bioactive products generated from arachidonic acid (see Fig 7-5) and generally have anti-inflammatory or proresolving properties. Lipoxins $A_4$ and $B_4$ ($LXA_4$ and $LXB_4$) are generated by the action of platelet-derived 12-lipoxygenase on neutrophil $LTA_4$.

It is now understood that the resolution of inflammation is an active instead of a passive process (ie, dilution of proinflammatory mediators) that is mediated by proresolving biochemical signaling circuits (Serhan 2007) (Fig 7-6a). During the initial stage of inflammation, eicosanoids


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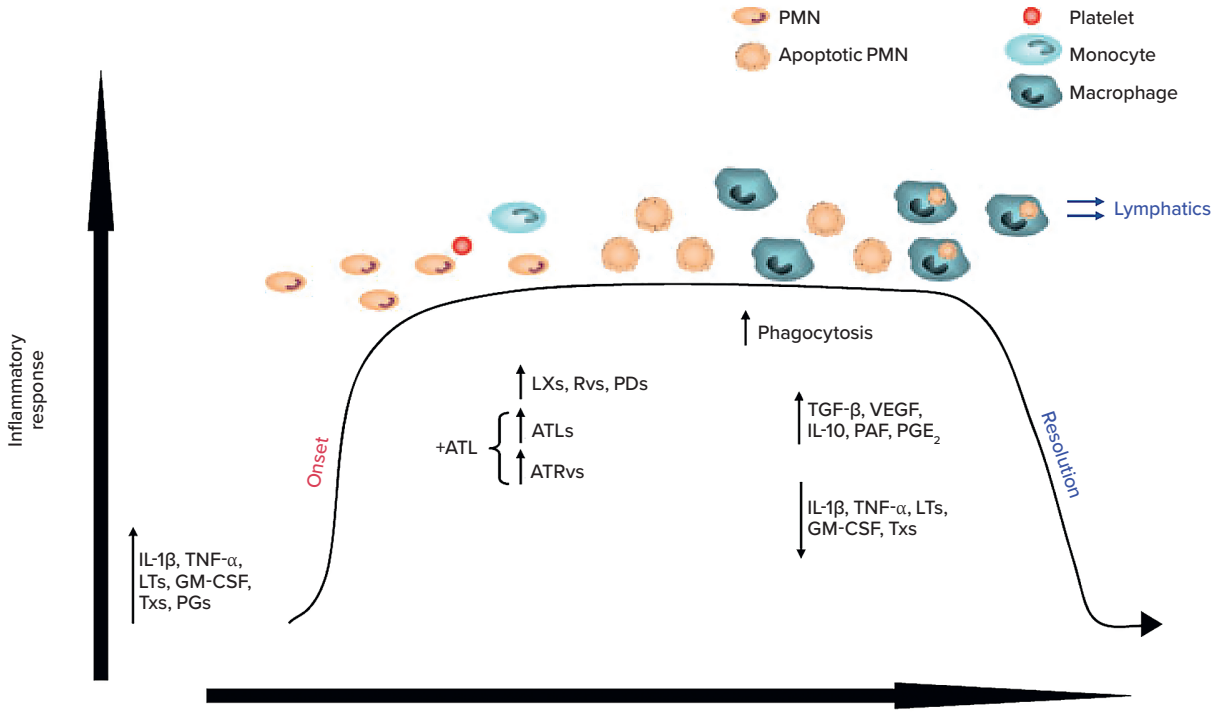


▲ **FIG 7-6a** Schematic illustration of the temporal cellular and biochemical events in the onset and resolution of inflammation. The initial phase of inflammation is characterized by the release of proinflammatory mediators and extravascular accumulation of neutrophils, followed by the infiltration of monocytes that differentiate into macrophages. This phase is followed by the formation of anti-inflammatory and proresolving mediators (lipoxins, aspirin-triggered resolvins, resolvins, and protectins). These mediators stop further neutrophil trafficking and facilitate the removal of apoptotic cells. The ingestion of apoptotic cells results in potent anti-inflammatory effects through the production of anti-inflammatory cytokines such as TGF-β1, IL-10, and $PGE_2$ and the limited release of proinflammatory mediators, including IL-8, TNF-α, and $TXA_2$. LTs = leukotrienes; Txs = thromboxanes; GM-CSF = granulocyte-macrophage colony-stimulating factor; PGs = prostaglandins; ASA = aspirin; LXs = lipoxins; Rvs = resolvins; PDs = protectins; ATLs = aspirin-triggered lipoxins; ATRvs = aspirin-triggered resolvins; PAF = platelet activating factor. (Reprinted with permission from Serhan 2007.)

including prostaglandins and leukotrienes play important roles as proinflammatory mediators, triggering potent chemotactic leukocytic responses. The second stage of inflammation is coupled to the biosynthesis of lipid mediators that actively limit inflammation and promote resolution (Fig 7-6b). These proresolving lipid mediators include endogenous lipoxins $LXA_4$ and $LXB_4$, their aspirin-triggered carbon-15 epimers (15-epi-$LXA_4$ and 15-epi-$LXB_4$), as well as resolvins, protectins, and maresins that are derived from omega-3 fatty acid precursors (Serhan 2008). Aspirin affects lipoxin generation, leading to the production of mediators known as *aspirin-triggered lipoxins* (ATLs). Thus, aspirin has a unique ability among the NSAIDs to initiate resolution of inflammation by "jump-starting" the early formation of proresolution lipid mediators that would normally be produced by leukocytes later in the inflammatory response.

Lipoxins are considered to act as "braking signals" in inflammation, limiting the trafficking of leukocytes to the inflammatory site. They also influence other target cell types (Fig 7-7) that are actively involved in the resolution of inflammation, stimulating phagocytosis of apoptotic cells by macrophages (Maderna and Godson). Lipoxins have emerged as potential antifibrotic mediators that may influence profibrotic cytokines and matrix-associated gene expression in response to growth factors. The rapid inactivation and short half-lives of lipoxins in vivo have led to the development of lipoxin analogs that are designed to resist metabolism and to preserve their structural integrity, bioavailability, and beneficial actions.

Traditionally used anti-inflammatory therapies have been directed toward the inhibition of enzymes and/or the antagonism of receptors. Both selective COX inhibitors and TNF-α antibodies are examples of this approach,

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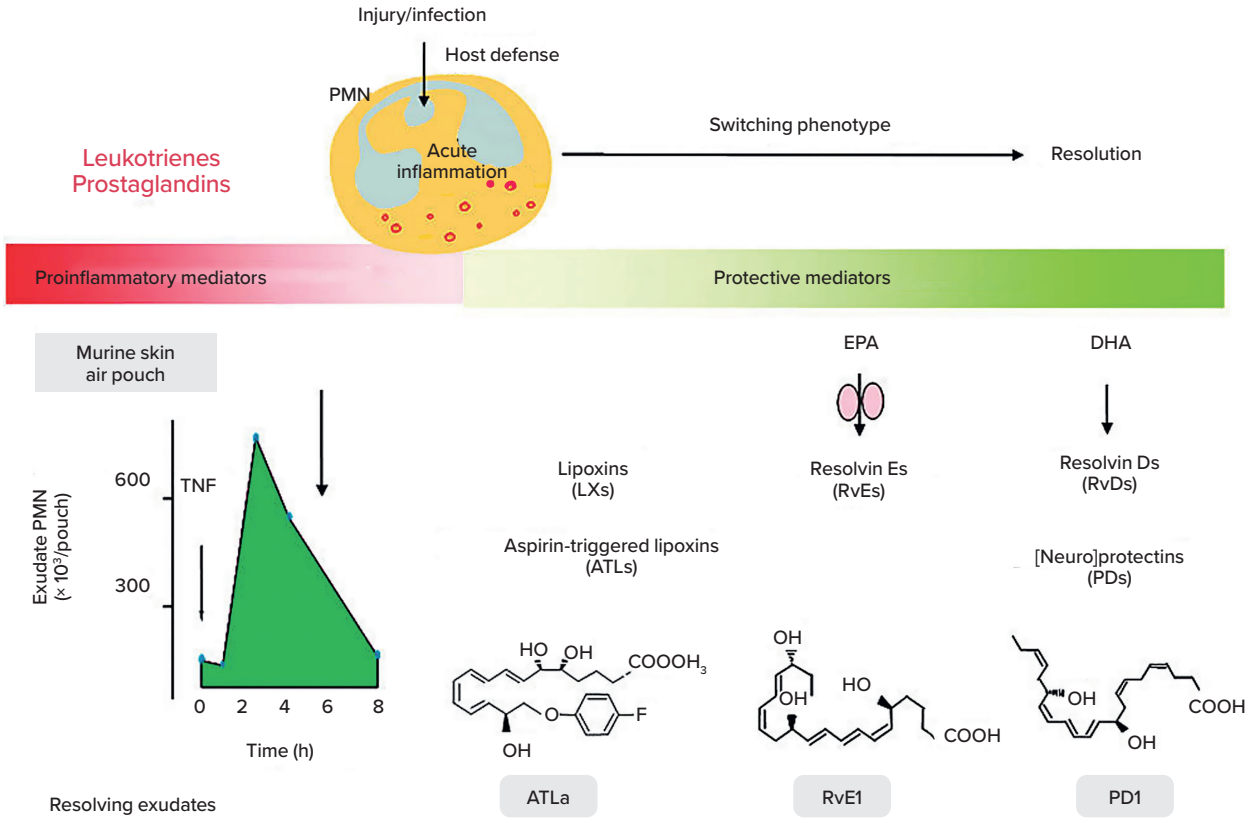


▲ **FIG 7-6b** Resolution of acute inflammation is an active process. In response to injury or infection, acute inflammation is normally a protective mechanism initiated by neutrophils. Neutrophil-derived proinflammatory mediators, such as leukotrienes and prostaglandins, can intensify this process, potentially leading to chronic inflammation. Within the resolution phase, neutrophils can promote inflammatory resolution by changing phenotype to generate protective mediators that are derived from polyunsaturated fatty acids (PUFAs). These mediators include lipoxins derived from arachidonic acid and aspirin-triggered epimer of lipoxins, resolvins derived from omega-3 PUFAs (eicosapentaenoic acid [EPA; resolving E series] and docosahexaenoic acid [DHA; resolvin D series]), and protectins enzymatically derived from DHA. (Reprinted with permission from Serhan 2008.)

and they are used with the goal of blocking production of proinflammatory chemical mediators. Specialized proresolving mediators (SPMs) have generated a paradigm shift in promoting the termination or resolution of acute inflammation (Aubeux et al). These effects have been demonstrated in endodontic and periodontal models (Siddiqui et al, Van Dyke).

## Specialized proresolving lipid mediators

Lipoxins were the first mediators recognized to have dual anti-inflammatory and proresolving activities. The SPMs include three distinct chemical families—resolvins, protectins, and maresins—that are involved in acute inflammation (Serhan 2009). Each of these families is actively biosynthesized from omega-3 precursors in the resolution phase of acute inflammation. These anti-inflammatory and proresolving lipid mediators are potent agonists that control the duration and magnitude of inflammation by acting on separate cell populations individually via the stimulation of specific receptors, thereby stimulating the overall resolution of inflammation.

### Resolvins

Resolvins are lipid mediators generated through the oxidation of the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Hence, there are E series resolvins (RvE) and D series resolvins (RvD) (Bannenberg). Resolvins were initially identified by harvesting in vivo exudates during the resolution phase of acute inflammation, defined by the time period of rapidly declining neutrophil cell numbers. Resolvins are also produced by a COX-2-dependent pathway in the presence of aspirin, generating their aspirin-triggered form. Resolvins possess potent anti-inflammatory and immunoregulatory actions that prevent excessive inflammatory


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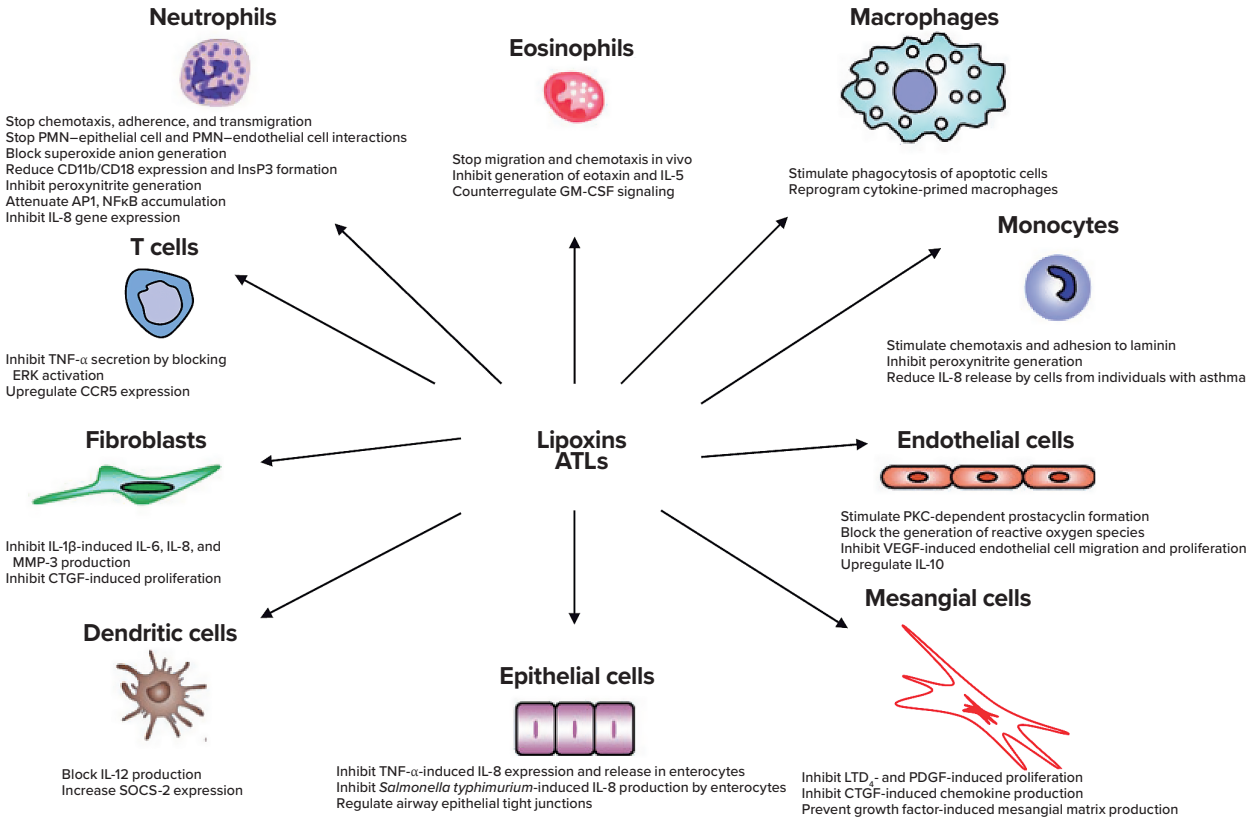
Neutrophils
Stop chemotaxis, adherence, and transmigration
Stop PMN–epithelial cell and PMN–endothelial cell interactions
Block superoxide anion generation
Reduce CD11b/CD18 expression and InsP3 formation
Inhibit peroxynitrite generation
Attenuate AP1, NFκB accumulation
Inhibit IL-8 gene expression
Eosinophils
Stop migration and chemotaxis in vivo
Inhibit generation of eotaxin and IL-5
Counterregulate GM-CSF signaling
Macrophages
Stimulate phagocytosis of apoptotic cells
Reprogram cytokine-primed macrophages
T cells
Inhibit TNF-α secretion by blocking ERK activation
Upregulate CCR5 expression
Monocytes
Stimulate chemotaxis and adhesion to laminin
Inhibit peroxynitrite generation
Reduce IL-8 release by cells from individuals with asthma
Fibroblasts
Inhibit IL-1β-induced IL-6, IL-8, and MMP-3 production
Inhibit CTGF-induced proliferation
Lipoxins ATLs
Endothelial cells
Stimulate PKC-dependent prostacyclin formation
Block the generation of reactive oxygen species
Inhibit VEGF-induced endothelial cell migration and proliferation
Upregulate IL-10
Dendritic cells
Block IL-12 production
Increase SOCS-2 expression
Epithelial cells
Inhibit TNF-α-induced IL-8 expression and release in enterocytes
Inhibit Salmonella typhimurium-induced IL-8 production by enterocytes
Regulate airway epithelial tight junctions
Mesangial cells
Inhibit LTD4- and PDGF-induced proliferation
Inhibit CTGF-induced chemokine production
Prevent growth factor-induced mesangial matrix production

▲ **FIG 7-7** Target cells for lipoxin $A_4$ and ATL bioactions. CD = cytoplasmic domain; CTGF = connective tissue growth factor; ERK = extracellular signal-regulated kinase; $LTD_4$ = leukotriene D4; MMP = matrix metalloproteinase; PDGF = platelet-derived growth factor; PKC = protein kinase C; SOCS-2 = suppressor of cytokine signaling-2. (Reprinted with permission from Maderna and Godson.)

responses and act via multiple cellular targets to stimulate resolution and preservation of immune vigilance (Serhan 2009). They block the production of proinflammatory mediators and regulate neutrophil trafficking to the inflammatory loci. The application of resolvin E1 (RvE1) has been shown to significantly reduce acute pulpal inflammation in vivo (Dondoni et al, Xu et al).

### Protectins

Like resolvins, protectins were also initially harvested in vivo from resolving inflammatory exudates. DHA also serves as the precursor for the biosynthesis of protectins. Protectin D1 (PD1), the most potent member, is synthesized from DHA via a lipoxygenase mechanism to a 10,17-dihydroxy-containing anti-inflammatory molecule. It is synthesized by human peripheral blood mononuclear cells and $T_h2$ $CD4^+$ T cells and blocks T-cell migration in vivo, reduces TNF-α and interferon-γ (IFN-γ) secretion, and promotes T-cell apoptosis. RvD1 and PD1 can serve as "braking" signals to prevent a runaway inflammatory response via several mechanisms such as the inhibition of neutrophil recruitment. Similar to RvE1, PD1 also shifts the onset of resolution to an earlier time point than spontaneous resolution. Both RvE1 and PD1 also upregulate CCR5 (see section on chemokines) on neutrophils, which acts as a "stop" signal to chemokine signaling by clearing proinflammatory CCR5 ligands from the inflammatory milieu (Kohli and Levy, Serhan 2009).

### Maresins

Maresins are produced exclusively by macrophages; they get their name from "**ma**crophage mediator in **res**olving **in**flammation." Maresins were also identified from self-resolving in vivo inflammatory exudates and possess dual anti-inflammatory and proresolving activities, with a potency similar to RvE1 and PD1. Maresin 1 can reduce

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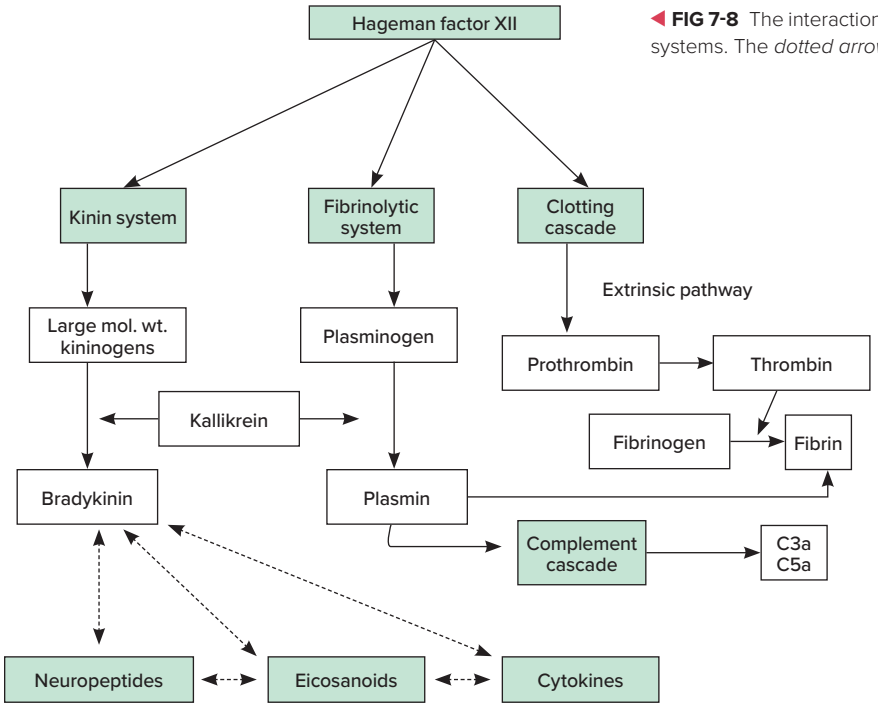


**◀ FIG 7-8** The interaction between selected groups of inflammatory systems. The *dotted arrows* indicate synergy and/or potentiation.

proinflammatory cytokines by inhibiting the NFκB pathway and activating the M2 macrophages, thereby reducing pain and enhancing regeneration (Dalli et al).

## Platelet activating factor

Platelet activating factor (PAF) is another bioactive mediator derived from phospholipids (see Fig 7-5). It is secreted by platelets, basophils (and mast cells), monocytes/macrophages, neutrophils, and endothelial cells. Its actions include platelet stimulation, leukocyte adhesion to endothelial cells by integrins, chemotaxis, and the production of an oxidative burst. It causes vasoconstriction in regular doses, but at very low doses it causes vasodilation that is 100 to 1,000 times more potent than histamine (Kumar et al). PAF stimulated the production of $PGI_2$ and $TXA_2$ in rat incisor pulp tissue ex vivo in a dose-dependent manner (Hirafuji and Ogura 1990). Furthermore, when the levels of these prostanoids were increased with the intravenous injection of endotoxin into the animals, the addition of a PAF antagonist suppressed the increase of $TXA_2$ (Hirafuji and Shinoda).

## Plasma proteases

### The kinin system and bradykinin

During the inflammatory process, prekallikrein is activated to kallikrein by the Hageman factor (coagulation factor XII) (Fig 7-8). Kallikreins, which are specific proteases, generate vasoactive peptides from plasma proteins called *kininogens*. The most important metabolite of the kininogens is the nonapeptide bradykinin (BK), which is the product of cleavage by plasma kallikrein (KLKB1), a serine protease. KLKB1 also activates plasminogen and other leukocyte and blood coagulation factors and processes proenkephalin, prorenin, and C3. KLKB1 was shown to induce inflammatory reactions in human dental pulp cells via protease-activated receptor 1 (Hayama et al).

BK has four main proinflammatory actions: vasodilation, increased vascular permeability, the induction of pain, and the attraction of leukocytes (Lewis). It functions by binding with two receptors: $B_1$ and $B_2$. $B_1$ appears to be involved in certain forms of persistent hyperalgesia or chronic pain, whereas the $B_2$ receptor is the main BK receptor constitutively present in normal tissues and plays a role in acute inflammatory pain (Hall). $B_2$ is also the principal BK receptor in the dental pulp (Sundqvist et al, Sundqvist and Lerner, Goodis et al 2000).

During pulpal inflammation, a complex interaction takes place between BK and several other molecular mediators, frequently eliciting synergy among the actions of the different mediators and an exaggeration of the inflammatory response. BK increased the release of arachidonic acid and its metabolites from a rat pulp cell line (Sundqvist et al). It also enhanced the formation of $PGE_2$ by IL-1α, IL-1β, TNF-α, and TNF-β (Sundqvist and Lerner). Using the bovine pulp superfusion model, $PGE_2$ was found to increase the release of BK-evoked iCGRP by more than 50% (Goodis et al 2000).


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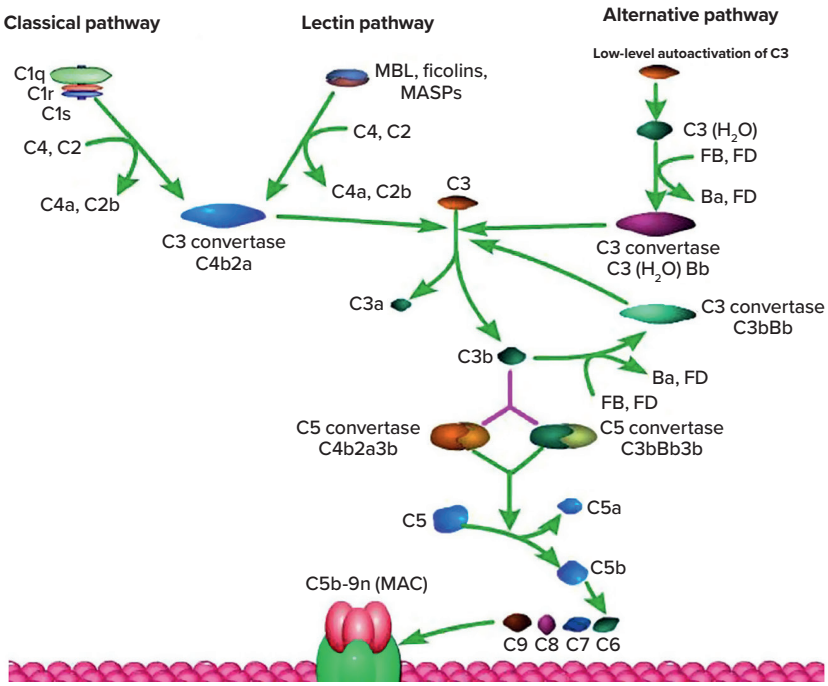


**FIG 7-9** The complement cascade. The complement system has three main complement activation pathways: the classical pathway (CP), the lectin pathway (LP), and the alternative pathway (AP). The CP is triggered by activation of the C1 complex (C1q–C1r–C1s). The LP is homologous to the CP and activated by mannose-binding lectin (MBL), ficolins, and mannose-binding protein-associated serine proteases (MASPs). The AP is continuously activated by low-level autoactivation of C3. All three pathways merge into a common terminal pathway with the activation of C5 into C5a and C5b. C5b binds to C6 and C7 and interacts with C8 and C9 to form the MAC. (Reprinted with permission from Zhang et al.)

Once released, BK is rapidly metabolized by specific kininases (Maita et al). Therefore, it has been difficult to measure BK levels in vivo. However, Lepinski et al were able to directly measure BK levels in the human pulp interstitial fluid using microdialysis. In that study, there was a 13-fold increase in BK level in irreversible pulpitis when compared with normal pulps. Patients who had pain at the time of sampling produced significantly more BK than those who had a previous pain history (17-fold increase vs 3-fold increase above normal levels). Anti-inflammatory drugs such as methylprednisolone (Hargreaves and Costello) or flurbiprofen (Swift et al) reduced the level of BK in inflamed tissues. Also, the addition of BK to homogenized rat pulp led to the release of the endogenous opiate met-enkephalin in a dose-dependent manner. Thus, homeostatic mechanisms may contribute to the control of BK release in vivo.

### Complement

The complement system consists of over 20 different plasma proteins (together with their cleavage products) that are utilized in both the adaptive and innate immune responses to initiate inflammation, aid in opsonization/phagocytosis, and cause lysis of microbial cells. Three complement activation pathways have been described: the classical, lectin, and alternative pathways (Fig 7-9). These pathways differ in the initial activation pathway and how C3b is produced but are similar after C3b production. Complement activation promotes phagocytosis because phagocytes express receptors for C3b. The terminal components of the complement system, whose activation is dependent on C3b, generate a lipid-soluble macromolecular protein complex called the *membrane attack complex* (MAC) that causes osmotic lysis of target cells. Peptides produced by proteolysis of C3 and other complement proteins stimulate inflammation. C3a and C5a, also known as *anaphylatoxins*, release histamine from mast cells and result in vasodilation and increased vascular permeability. C5a activates the lipoxygenase pathway of arachidonic acid in neutrophils and monocytes and is itself a potent chemotactic agent for these cells. Earlier studies revealed weak complement activity in the dental pulp (Pulver et al, Speer et al, Pekovic and Fillery). More recently, pulp fibroblasts were shown to produce a MAC that killed oral streptococci (Jeanneau et al) and controlled nerve regeneration (Chmilewsky et al). In addition, complement activation was shown to mediate pulp stem cell activation and differentiation (Rufas et al, Irfan et al, Pasiewicz et al) and to play a critical role in phagocytosis, chemotaxis, and dental tissue regeneration (Le Fournis et al, Bergmann et al).

### The clotting and fibrinolytic systems

The clotting system is closely related to the inflammatory process. As discussed previously, prekallikrein is activated to kallikrein in inflammation by the action of the Hageman factor XII, which also starts the extrinsic pathway of the coagulation cascade (see Fig 7-8). Several intermediary

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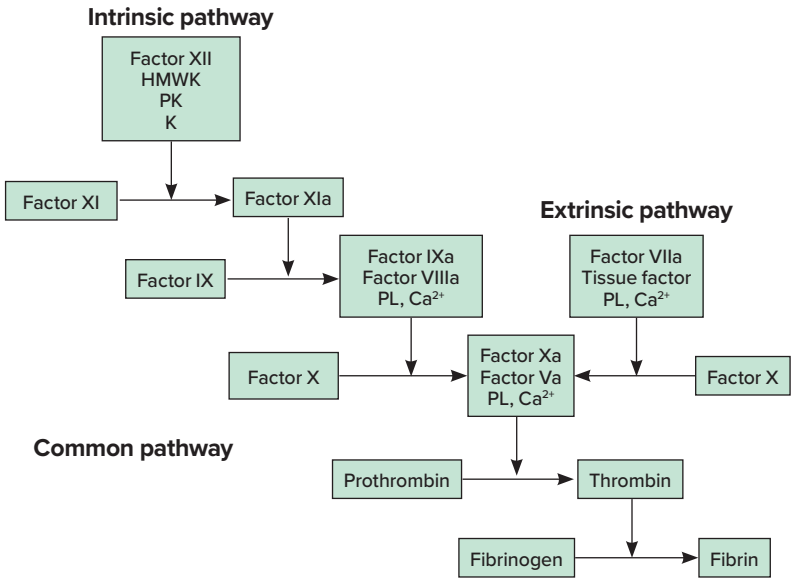
steps result in the formation of thrombin (factor IIa, a serine protease) from prothrombin. In addition to activating fibrinogen to form fibrin, activating factor XIII to cross-link the fibrin polymer, and aggregating platelets, thrombin has several inflammatory properties such as chemotaxis, leukocyte adhesion to endothelial cells, and fibroblast proliferation. The addition of thrombin to pulp fibroblasts caused a burst in the production of $PGE_2$ and 6-keto-$PGF_{1\alpha}$, an effect that was very similar to the addition of BK (Sundqvist et al). Thrombin increases DNA and protein synthesis and the proliferation of pulp fibroblasts, and these effects can be modulated by $PGE_2$ (Chang et al 1998a, Chang et al 1998b, Chang et al 1999).

Traditionally, the coagulation process is represented by a Y-shaped cascade of proteolytic reactions that act as a biologic amplifier, with the extrinsic and intrinsic pathways meeting in a common pathway (Fig 7-10). While this coagulation cascade provides the framework for interpreting the results of common coagulation screening tests in plasma, where the fluid is static and does not interact with vascular wall or cell surfaces, it has severe limitations as a clinical hemostasis model. The classical cascade model of hemostasis has now been replaced by the cell-based model of coagulation, which incorporates the vital role of cells in the coagulation process. The cell-based model requires the participation of two different cell types: a cell-bearing surface tissue factor and platelets. The cell-based model highlights the central role of thrombin (factor IIa) in hemostasis. This new model further suggests that there are no separate extrinsic and intrinsic pathways operating under normal conditions in vivo. The extrinsic and intrinsic systems are in fact parallel generators of factor Xa that occur on different cell surfaces instead of being independent redundant pathways (Romney and Glick).

In the context of the present chapter, the cell-based model further incorporates the concept that some coagulation proteases, while involved in the enzymatic cascade of coagulation, may function primarily in roles apart from hemostasis, including inflammation, vessel wall function, and cell proliferation. For example, factor XII (Hageman factor) also initiates the fibrinolytic system to solubilize the fibrin clot. A plasminogen activator is secreted from leukocytes and endothelial cells to cleave plasminogen into plasmin, a protease. Plasmin's main action is to lyse the fibrin clot. However, it also breaks C3 into its fragments, aiding in the initiation of the complement cascade (see Fig 7-8). Fibrinolytic activity was demonstrated in the dental pulp several decades ago (Southam and Moody). A fibrin clot is formed under cavity preparations, particularly if the pulp is exposed (Mjör et al, Sasaki and Kawamata-Kido); fibrinogen has been localized in the pulp and inside dentinal tubules under cavity preparations when the pulp was not exposed (Wikström et al, Izumi et al) and may reduce dentinal permeability to advancing microbial irritants (Pashley et al). Tissue-type plasminogen activator was shown to increase significantly in inflamed pulp at the gene expression and protein levels (Huang et al 2005), being upregulated in the presence of proinflammatory cytokines (Huang et al 2005, Huang et al 2006). Furthermore, platelet-rich fibrin and platelet-rich plasma have been used in regenerative endodontic therapy due to their effect on odontoblastic differentiation of pulp stem cells, in addition to other growth factor content (Woo et al, Kim et al 2017, Chai et al).




◀ **FIG 7-10** The classical cascade of coagulation. This model divides the coagulation system into separate redundant pathways (extrinsic and intrinsic), either of which can result in generation of factor Xa. The common pathway results in the generation of thrombin (factor IIa) and subsequent cleavage of fibrinogen to fibrin. Many of the enzymes and enzymatic complexes require calcium ($Ca^{2+}$) and binding to phospholipids (PL) on active membrane surfaces for full activity. For simplicity, feedback activation of procofactors to cofactors and the many inhibitors of the various enzymes have been omitted. HMWK = high molecular weight kininogen; K = kallikrein; PK = prekallikrein.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### Lysosomal enzymes and metalloproteinases

Neutrophils and monocytes/macrophages have lysosomal granules containing several enzymes, which contribute to the inflammatory process. Neutrophils contain two types of granules; the smaller or specific granules contain lysozyme, collagenase, lactoferrin, plasminogen activator, histaminase, and alkaline phosphatase, whereas the larger azurophilic granules contain myeloperoxidase, lysozyme, defensins, acid hydrolases, and neutral hydrolases such as matrix metalloproteinases (MMPs; including collagenase), elastase, cathepsin G, and other proteinases. Most of these enzymes have potent antimicrobial properties, thus serving in eliminating microbial irritants. However, they can also lead to excessive tissue destruction during an inflammatory episode. Excessive neutrophil accumulation in the pulp may increase the likelihood of tissue necrosis. Cathepsin G together with elastase and lactoferrin were shown to increase during pulpal inflammation (Rauschenberger et al 1991, Cootauco et al, Rauschenberger et al 1994). The protease inhibitor $\alpha_2$-macroglobulin was also observed to increase with inflammation, indicating an attempt to control the tissue-destruction aspect of these enzymes (McClanahan et al). The intensity of pulpal inflammation may determine the neutrophil/monocyte infiltration, their release of lysosomal enzymes, and the outcome of the balance between inflammation and regeneration.

MMPs are members of an enzyme family that require a metallic ion (eg, zinc) in their active site for catalytic activity (Murphy and Nagase, Kumar et al). MMPs are critical for maintaining tissue allostasis (tissue response to stressors for regaining homeostasis). They are active at neutral pH and can therefore catalyze the normal turnover of ECM macromolecules such as the interstitial and basement membrane collagens; proteoglycans such as aggrecan, decorin, biglycan, fibromodulin, and versican; as well as accessory ECM proteins such as fibronectin. The human genome has 24 matrixin genes, which include a duplicated Mmp23 gene. Thus, there are 23 members in the human MMP family, including the "classical" MMPs (collagenases MMP-1, -8, and -13; and gelatinases MMP-2 and -9), stromelysins (MMP-3 and -10), matrilysins (MMP-7, -11, and -26), the membrane-bound MMPs (MT-MMPs; MMP-14, -15, -16, -17, -24, and -25), and other MMPs (MMP-12, -19, -20, -21, -23, -27, and -28). Most of the MMPs are synthesized as inactive latent enzymes. Conversion to the active enzyme is generally mediated by activator systems that include plasminogen activator or the prohormone convertase, furin. More recently, the ADAMs (a disintegrin and metalloproteinase) (Edwards et al) and the ADAMTSs (a disintegrin and metalloproteinase with thrombospondin motif) (Porter et al) were identified. Functional ADAMs are involved in "ectodomain shedding" of diverse growth factors, cytokines, receptors, and adhesion molecules. An important ADAM family member (ADAM-17) is a TNF-α–converting enzyme that activates pro-TNF-α. ADAMTSs are extracellular, multidomain enzymes. Their known functions include collagen processing as procollagen N-proteinase; cleavage of the matrix proteoglycans aggrecan, versican, and brevican; and inhibition of angiogenesis.

Interstitial collagenase (MMP-1) was detected in ameloblasts and odontoblasts of the developing enamel organ (Caron et al). Gelatinases (MMP-2 and -9) were found in the dentinoenamel junction (Goldberg et al 2002) and in ameloblasts, odontoblasts, and pulp of rodent incisors (Goldberg et al 2002). Anther collagenase (MMP-13) was found to be present in equivalent amounts in the pulp of normal and carious teeth (Sulkala et al 2004). Enamelysin (MMP-20) was first detected in the enamel organ and dental papilla but was also found in the pulp of mature teeth with or without caries (Sulkala et al 2002). An extensive analysis of the presence and upregulation of MMPs in odontoblasts and the dental pulp tissue revealed that MMP-1, -2, -9, -10, -11, -13, -14, -15, -16, -17, -19, -20, and -23 were expressed by both odontoblasts and pulp tissue. MMP-7, -8, -24, and -25 were expressed only in odontoblasts. MMP-2, -10, -11, -14, and -20 were expressed more abundantly by odontoblasts, whereas pulp tissue expressed more MMP-13 and -17. Transforming growth factor β1 (TGF-β1) alone or with bone morphogenetic protein 2 (BMP-2) significantly upregulated MMP-9 but not MMP-20 mRNA in odontoblasts; however, in pulp tissue no effects could be detected (Palosaari et al).

Host MMP-2, -8, and -9, probably of salivary rather than pulpal origin, participate in the degradation of demineralized dentin under a caries lesion (Tjäderhane et al). MMP-2, -8, and -9 were detected in human radicular dentin (Santos et al) and may have significance in degrading hybrid layers created by contemporary resin-based root canal sealers. Clearly, MMPs may contribute to the remodeling of dentin and pulp that takes place in physiologic and pathologic situations. MMP-9 levels in dentinal fluid are significantly increased in cases of irreversible pulpitis (Zehnder et al 2011) and correlate with caries depth (Ballal et al 2017), indicating that this enzyme—or other cytokines and growth factors (Brizuela et al) —may be used as markers of significant pulpal inflammation in caries lesions with unexposed pulp.

In cases with irreversible pulpitis, MMP-2 and MMP-3 were found to be upregulated in one study (Shin et al) but downregulated in another study (Gusman et al). MMP-3 was shown to have anti-inflammatory effects in a model of inflamed pulp (Eba et al). Together with SP, NKA, and IL-8, MMP-8 levels were significantly increased in the pulp and crevicular fluid in cases with irreversible pulpitis

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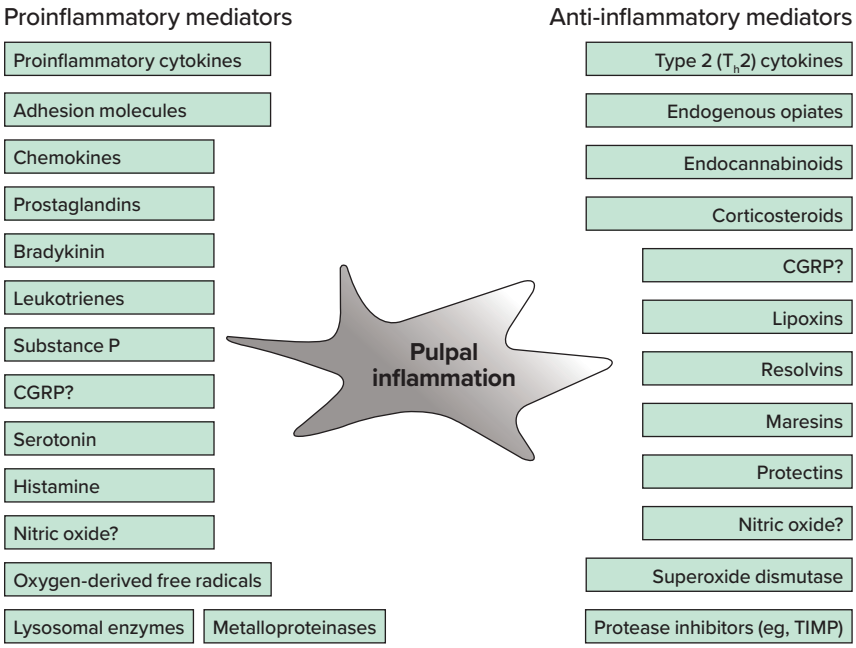


◀ **FIG 7-11** Important proinflammatory and anti-inflammatory mediators of pulpal inflammation.

compared to controls, and the levels in crevicular fluid decreased 1 week after pulpectomy in these cases (Akbal Dincer et al). Interestingly, it has also been demonstrated that painful stimulation of teeth induces an increase in MMP-8 levels sampled from their crevicular fluid (Avellan et al). MMP-9 is also significantly increased in irreversible pulpitis (Gusman et al, Tsai et al, Mente et al). Furthermore, it has recently been demonstrated that MMP-9 is predictive of the outcome of dental pulp capping (Ballal et al 2022) and of pulpotomy (Sharma et al). Recognizing that symptomatic irreversible pulpitis is very arbitrarily defined in different studies (Mejare et al) and that there are many other inflammatory mediators expressed in pulpitis (Rechenberg et al 2016, Kritikou et al), newer data is emerging to investigate many of these proteins and their associations with specific signs and symptoms. In this regard, one preliminary study showed high correlations of pulpal MMP-1, -2, -9, and -12; RANTES (regulated on activation, normal T-cell expressed and secreted); macrophage inflammatory protein-2 (MIP-2); MCP-1; and P-selectin with presenting pain level and duration of pain to cold (Fouad 2022).

One study showed that ADAM28 may be involved in the proliferation, differentiation, and apoptosis of human DPSCs (Zhao et al). Another more recent study showed that ADAMTS-1 and -9 were significantly increased in inflamed pulp compared with healthy pulp, whereas levels of tissue inhibitor of metalloproteinase-3 (TIMP-3; see below) decreased (Golbasi et al).

### Protease inhibitors

As is typically the case, the inflammatory process (and occasionally developmental processes) involves proinflammatory and anti-inflammatory mediators in a balance that depends on the amount of irritation and mediates eventual development, homeostasis, and/or regeneration (Nakata et al) (Fig 7-11).

Protease inhibitors serve the important function of limiting the normal protease, including metalloproteinase, defense functions that may damage the host tissue if they are not regulated. The balance between activated MMPs and the so-called *tissue inhibitors of metalloproteinases* (TIMPs; TIMP-1, -2, -3, -4) controls the extent of ECM remodeling (Murphy and Nagase). TIMP-1, -2, and -3 were found to be expressed by both odontoblasts and pulp tissue (Palosaari et al). MMP-2, MMP-9, TIMP-1, and TIMP-9 were shown to be present during tooth development (Goldberg et al 2003).

Experimental models for pulpitis show an increase in TIMP-1 with pulpitis (Li et al 2021). Furthermore, several clinical studies showed increased levels of TIMP-1 in the dentinal fluid (Brizuela et al) or pulp (Mente et


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al) of patients with inflamed pulp compared to healthy controls, and MMP-9 and active MMP-2 were accompanied by significant increases in TIMP-2 and myeloperoxidase (MPO) (Accorsi-Mendonca et al) in inflamed pulp compared with healthy tissues.

## Nitric oxide and oxygen-derived free radicals

Despite its high reactivity and short half-life, nitric oxide (NO) contributes to a large array of biologic functions. NO is a soluble gas that was first identified due to its action of relaxing smooth muscle, causing vasodilation. This effect was also shown in the dental pulp (Lohinai et al 1995, Berggreen and Heyeraas 1999). It may also have antimicrobial properties. NO is synthesized via L-arginine oxidation by a family of NO synthases (NOSs) and several cofactors including nicotinamide adenine dinucleotide phosphate (NADPH). Three different isoforms of the NOS enzyme exist: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS) (Kumar et al). While nNOS and eNOS are constitutively expressed in the respective tissues and are calcium dependent, iNOS is induced in macrophages and several other cells, primarily by cytokines (such as IL-1 and TNF-α) or microbial products (such as LPS), and are calcium independent. The cytokines IL-4, IL-10, and TGF-β regulate the expression of iNOS in macrophages (Billiar). Depending on the site of production, the amount of NO produced, and the targets within the local environment, NO can exert very different effects. A small quantity of NO released by the vascular endothelium regulates the relaxation of adjacent smooth muscle and protects against the adhesion of leukocytes and platelets to the blood vessel wall. These properties may be considered protective and "anti-inflammatory" (see Box 7-1). In contrast, the much larger amounts of NO released by cells in response to cytokines can destroy host tissues and impair discrete cellular responses. Finally, by affecting functions of lymphocytes and macrophages, induced NO can exert an immunomodulatory role that modifies the course of disease (Clancy et al) (see Box 7-1). Experimental models of pulpitis show increased production of iNOS together with other inflammatory mediators like IL-6, IL-1β, TNF-α, CCL2, CXCL1, CXCL2, and MMP-9 (Renard et al).

nNOS has been demonstrated in feline teeth (Lohinai et al 1997). There was a dramatic increase in NO activity with pulpal inflammation, as evidenced by immunoreactivities to NOS and the NADPH cofactor at the site of pulp irritation. These activities increased to a maximum at 4 days postoperatively and declined at 14 days with evidence of necrosis. In human pulp, eNOS was identified in the endothelial cells and odontoblasts of healthy tissues, and an elevation of eNOS mRNA and protein with a concomitant dilation of vessels was characteristic in inflamed tissue (Di Nardo Di Maio et al). Healthy pulp tissue failed to exhibit any iNOS; however, acute inflammation enhanced the mRNA and protein levels of iNOS, mainly in the leukocytes (Di Nardo Di Maio et al). Pretreatment of pulp with the NOS inhibitor NG-nitro-L-arginine methyl ester (L-NAME) in vivo did not affect vasodilation but significantly potentiated SP-mediated vasodilation, possibly via increased activity of the enzyme guanylate cyclase (Hsu et al). Likewise, SP induced NO production by activating NOS in pulpal endothelial cells (Karabucak et al).

Reactive nitrogen and oxygen species have overlapping actions. This was shown by knockout mice experiments in which loss of phagocyte oxidase or iNOS was only mildly susceptible to infection, whereas animals lacking both rapidly succumbed to disseminated infections by commensal microorganisms (Kumar et al).

The production of reactive oxygen species (ROS) by mammalian mitochondria is important because it underlies oxidative damage in many pathologies and contributes to retrograde redox signaling from the organelle to the cytosol and nucleus (Murphy).

Oxygen-derived free radicals (ODFRs) are potent inflammatory mediators released from neutrophils that are challenged with antigen-antibody complexes or chemotactic agents. The superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide ($H_2O_2$), and the hydroxyl radical ($OH^{\bullet}$) are the most important species. These can combine with NO to form peroxynitrite and other toxic nitrogen intermediates. ODFRs, also known as *reactive oxygen intermediates*, upregulate the production of IL-8, and antioxidants are known to reduce this cytokine (Remick and Villarete). The enzyme superoxide dismutase (SOD) is the main intracellular scavenger of ODFRs, particularly $O_2^{\bullet -}$. It has been demonstrated that the $OH^{\bullet}$ radical reduces PBF (Okabe). This may be due to its effect on the endothelium or could follow the rise in tissue pressure in the low-compliance pulp environment that accompanies pulpal inflammation. Copper and zinc-containing SOD (CuZnSOD) is found in the cytoplasm, whereas manganese-containing SOD (MnSOD) is located in the nucleus or the mitochondria. In humans, CuZnSOD activity was identified in low quantities in normal pulp but increased significantly in inflamed pulp (Davis et al). In the latter study, it was noted that the enzyme activity decreased with advancing age of the patient, in both the normal and inflamed pulp groups. A clinical study has revealed that catalase, an enzyme that reduces the highly reactive-oxygen molecule $H_2O_2$, increases significantly in reversibly inflamed pulp—an increase that is moderated as the inflammation is diagnosed to be irreversible (Esposito et al). Areas of intense inflammation and with leukocytic infiltrates in rat pulp

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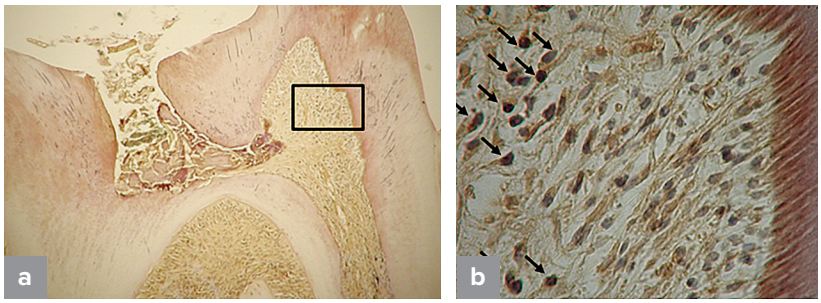


◀ **FIG 7-12** Mouse molar with pulp exposure in the mesial pulp horn. The sample was stained for IL-1α 1 week after pulp exposure. The distal portion of the chamber and the pulp in the distal canal showed an inflammatory infiltrate. *(a)* Low magnification showing pulp exposure (original magnification ×100). *(b)* High magnification showing IL-1α-positive cells (original magnification ×1,000).

are associated with a dramatic increase in levels of both CuZnSOD and MnSOD activities (Baumgardner et al).

The levels of ROS increase following external vital dental bleaching (Sato et al, Benetti et al, Chen et al 2021) or pulp irritation due to resin monomers (Schweikl et al) but are reduced together with NO with the application of tricalcium silicate cements (Chang et al 2014). Moreover, replicative senescence (aging) of human dental pulp cells (Lee et al 2013c) and induced diabetes mellitus (Leite et al) were characterized by increases in ROS. Finally, N-acetyl cysteine was found to reverse the production and harmful effects of ROS on human pulp cells (Jiao et al).

## Cytokines

The inflammatory response represents a closely regulated balance between proinflammatory and anti-inflammatory mediators (see Fig 7-11) that is titrated to neutralize the harmful effects of the advancing irritant while minimizing damage to host tissues (Fig 7-12). This concept is very well illustrated by a discussion of cytokines in pulpal inflammation (Fig 7-13). Cytokines are proteins released from cellular components in an inflammatory process that activate, mediate, or potentiate actions of other cells or tissues. Their actions can be achieved by an autocrine (self-activating), paracrine (acting locally), or endocrine (acting systemically) manner. While most of the cytokines present in an inflammatory process are produced by inflammatory cells like monocytes/macrophages, lymphocytes, and neutrophils, they may also be produced by several noninflammatory cells, which in the dental pulp would include odontoblasts, fibroblasts, and endothelial cells. Cytokine secretion is a brief, self-limited event initiated by new gene transcription with messenger RNA encoding that is transient in the activated cell. Cytokines have numerous overlapping, and sometimes seemingly redundant, functions that upregulate either proinflammatory or anti-inflammatory activities, resulting in a closely regulated, well-orchestrated inflammatory process.

Cytokines play a central role in pulpal inflammation (Elsalhy et al, Hirsch et al). In this regard, there have been several studies that investigated therapeutic agents that target cytokines for the treatment of pulpitis (Khorasani et al). There are two main classifications of cytokines. A structural classification addresses the molecular structure and the types of cells that produce the cytokine; eg, type I cytokines share a four-α-helical structure and receptor structure and are produced by $T_h1$ cells, and type II cytokines are produced by $T_h2$ lymphocytes. A functional classification describes their role in inflammation in that they are either proinflammatory, anti-inflammatory, or effectors of chemotaxis or chemokines. A modification of the latter classification is presented here.

### Proinflammatory cytokines

Cytokines secreted by innate immune cells from the dental pulp have been comprehensively reviewed (Fouad and Huang). Among the most important proinflammatory cytokines are IL-1 and TNF-α. The actions of these two cytokines are very similar even though they interact with structurally different receptors. IL-1 is expressed in two isoforms: IL-1α and IL-1β. IL-1 is produced mainly by monocytes/macrophages but may also be produced by polymorphonuclear lymphocytes (PMNs), fibroblasts, and endothelial cells. IL-1 has several systemic effects such as the production of fever, acute phase proteins, prostaglandins, platelet activation factor, and nitric oxide. Locally, IL-1 activates T cells and stimulates them to produce IL-2 and induces the synthesis of prostaglandins. IL-1 and TNF-α also activate endothelial cells and induce the expression of adhesion molecules on their membranes, thereby aiding in the recruitment of inflammatory cells to the site of inflammation. In the pulp, it inhibits proliferation of pulpal fibroblasts and induces the expression of collagenase from pulpal fibroblasts.

IL-1 and TNF-α are heavily expressed in pulpal inflammation (see Fig 7-11) (Abd-Elmeguid et al, Hirsch et al, Wu et al). The production of IL-1 and TNF-α in the pulp


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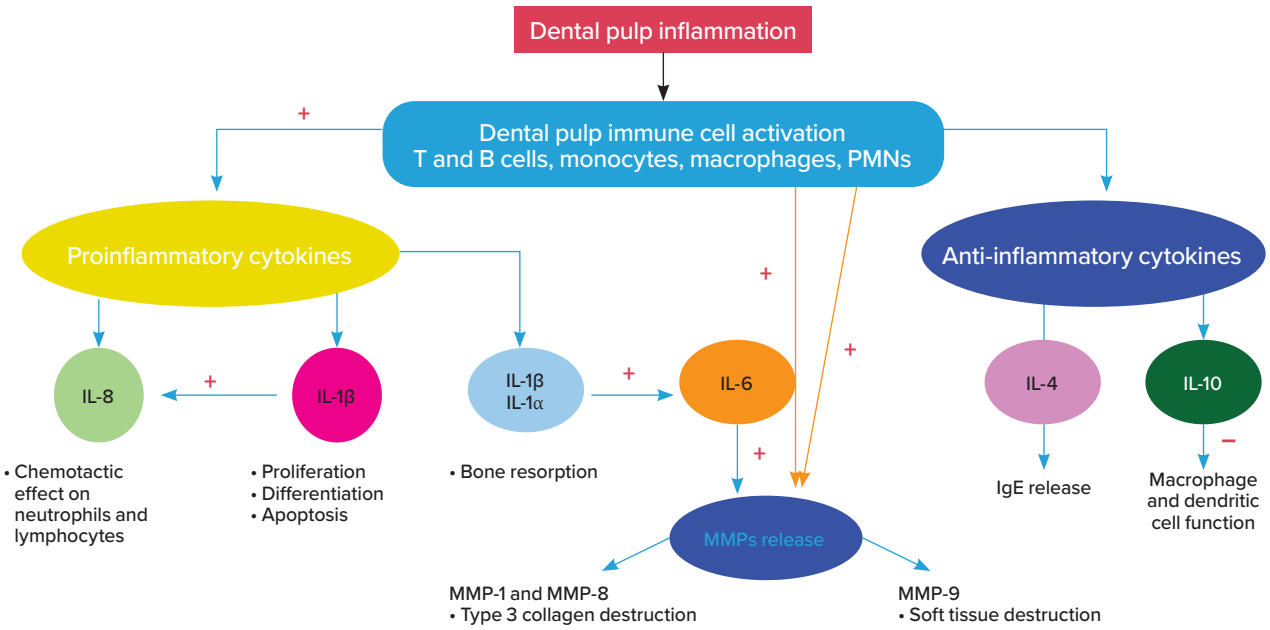
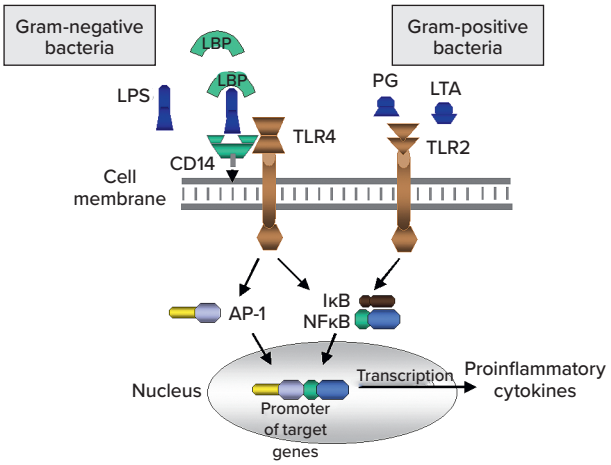



▲ **FIG 7-13** IL-MMP interactions and their molecular consequences in the context of pulpal inflammation. (Based on Kritikou et al.)

is probably a result of direct irritation by bacterial virulence factors such as cell wall products. The progression of pulpal inflammation and the tissue expression of IL-1β and TNF-α were shown to be comparable in normal mice and mice that lacked any functional T or B cells, indicating that the production of these cytokines could occur without the presence of adaptive immunity (Fouad 1997). LPS from *Porphyromonas endodontalis* applied to human dental pulp cells in vitro produced IL-1β in a dose-dependent manner (Hosoya and Matsushima). Peptidoglycans (PG) and lipoteichoic acid (LTA) from gram-positive bacteria were shown to have a similar, albeit less potent, effect on the production of IL-1 and TNF-α in other systems (Mattsson et al) (Fig 7-14). LPS achieves its effect by binding initially to an LPS-binding protein (LBP) in the blood. LBP attaches to cell surface receptors on macrophages called *CD14*. These receptors do not have a transmembrane domain to effect intracellular transduction of the signal.




▲ **FIG 7-14** Pathway for proinflammatory cytokine production after stimulation by bacterial cell wall products.

The relative importance of IL-1 and TNF-α in mediating pulpal responses to mixed bacterial infection was investigated in a mouse model (Chen et al 1999). Pulp exposures were created in mice deficient in IL-1 receptor, TNF p55 and p75 receptors, or both IL-1R and TNF p55 receptors (dual deficiency). The receptor-deficient mice had a significantly faster rate of pulpal degeneration and microbial penetration than the wild type mice, and the animals with dual deficiency had worse results than those with either deficiency alone. These findings indicate that despite the proinflammatory properties of these cytokines, they have a protective role in the dental pulp against the spread of infection. It is important to recognize that the production of IL-1 and TNF-α from pulpal macrophages in response to bacterial irritants may be suppressed by the toxic effects of dental filling materials that may come in contact with the pulp (Rakich et al 1998, Rakich et al 1999). An analysis of the gene expression profile in normal compared to inflamed symptomatic human pulp showed that IL-1α and IL-1β were not significantly increased in some studies (Zehnder et al 2003) but were

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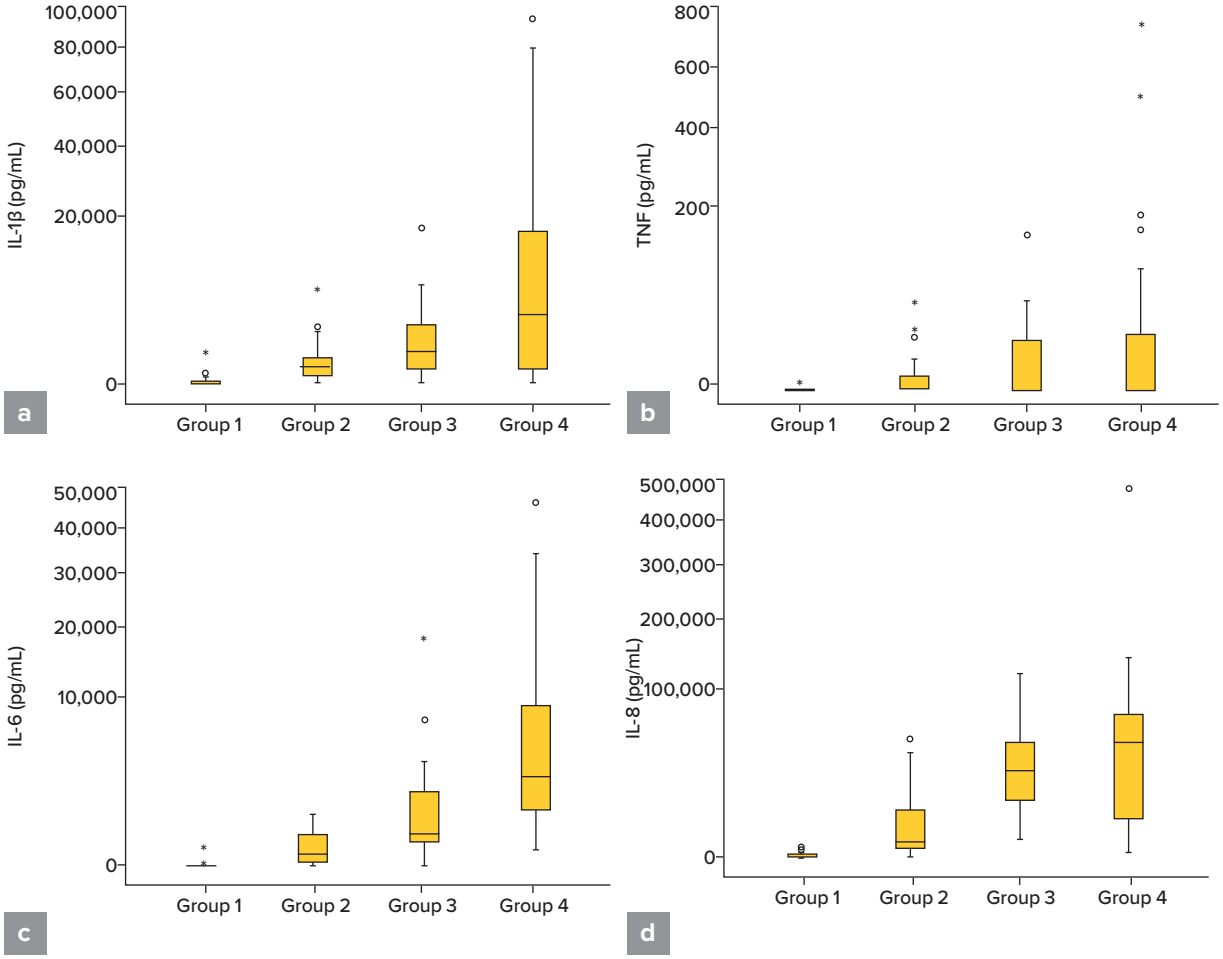
increased in others (Galicia et al). Studies have also shown that IL-6 (which has both pro- and anti-inflammatory effects), IL-8 (a chemokine), and IL-18 (a proinflammatory cytokine with a similar profile to IL-12) were all significantly increased in painful pulpitis (Zehnder et al 2003, Galicia et al, Al Natour et al 2021b). These findings were corroborated in a recent protein expression study in which the stages of caries progression and the development of symptoms were evaluated separately (Wu et al) (Fig 7-15). Interestingly, in this study, only IL-6 was significantly increased in cases with deep caries lesions that were symptomatic compared to deep caries lesions that had mild or no symptoms.

IL-12 is a cytokine produced by monocytes, macrophages, and dendritic cells, and it is thought to be essential for $T_h1$ cell differentiation and proliferation. It is also essential in the production of IFN-γ by $T_h1$, $CD8^+$, γδ T cells, and natural killer (NK) cells (Fouad and Huang). Peripheral blood mononuclear cells challenged with *Streptococcus mutans*, which is associated with superficial caries, produced significantly more IFN-γ and IL-12 than those challenged with *Lactobacillus casei*, which is associated with deep caries (Hahn et al 2000).

IL-17 is another proinflammatory cytokine that is highly expressed in irreversible pulpitis; it induces the production of IL-6 and IL-8 and activates NFκB, phosphorylation of p38 mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK) in dental pulp cells (Xiong et al). Its production is mediated by WNT5A (member of WNT, Wingless type, proteins that have been implicated in oncogenesis and development of various tissues) (Liu et al 2019). IL-17 leads




▲ **FIG 7-15** Levels of three proinflammatory cytokines—*(a)* IL-1β, *(b)* TNF, and *(c)* IL-6—and *(d)* chemokine IL-8 (CXCL8) in patients with normal pulps (group 1), early-stage chronic pulpitis (shallow caries; group 2), late-stage chronic pulpitis (deep caries with mild symptoms; group 3), and acute attack of chronic pulpitis (symptomatic deep caries; group 4). Of note, only groups 3 and 4 had significantly different levels of IL-6, and TNF levels in groups 2, 3, and 4 were not significantly different. (Data from Wu et al.)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to the production of another proinflammatory cytokine, IL-23 (Wei et al 2018), which causes further differentiation and expansion of $T_h17$ cells (Arora et al, Abbas et al).

T helper 1 ($T_h1$) cytokines are generally considered to be proinflammatory cytokines. These cytokines include IL-2, IL-12 (α and β), and IFN-γ. These cytokines are mostly involved in cell-mediated hypersensitivity and self-activation of T cells (IL-2) as well as activation of B cells and macrophages (IFN-γ). IFN-γ augments TNF-α activity and induces NO release (Dinarello). It is noteworthy that IFN-γ is produced by NK cells in addition to being a $T_h1$ cytokine. This finding may explain the production of proinflammatory cytokines in T-cell–deficient mice and illustrate the multitude of cellular sources for key inflammatory cytokines. IL-2 levels were significantly elevated in histologically verified cases of symptomatic irreversible pulpitis (Rauschenberger et al 1997) but similar to levels in normal and necrotic pulp in another study (Anderson et al). More recent studies show elevation of this important cytokine in pulpitis cases (Abd-Elmeguid et al, Elsalhy et al, Hirsch et al). Shallow caries lesions mostly colonized by *S mutans* were shown to elicit a strong type 1 (proinflammatory) cytokine response (Hahn et al 2000). The expression of mRNA for IFN-γ in human pulp samples was found to be significantly higher than that of IL-10 and IL-4 and significantly greater in superficial compared to deep caries (Hahn et al 2000, Hahn et al 2004).

#### Immunoregulatory or anti-inflammatory cytokines

$T_h2$ cells produce IL-4, -5, -6, -9, -10 (also secreted by $T_h1$ and macrophages), and -13. Both $T_h1$ and $T_h2$ produce IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF). $T_h2$ cytokines also serve the very important function of inhibiting most of the proinflammatory functions caused by other cytokines, such as IL-1, IL-12, and IFN-γ, thus maintaining the homeostasis of the immune response. This latter function is primarily regulated by IL-10, -4, and -13. Peripheral blood mononuclear cells challenged with *S mutans* generated a dose-dependent increase in IL-10 production but no IL-4 production (Hahn et al 2000). The levels of mRNA expression were significantly lower for IL-10 and IL-4 compared to IFN-γ in shallow caries lesions, but this difference disappeared in deep caries lesions (Hahn et al 2000). There is probably an increased need for these two immunoregulatory cytokines in deep caries lesions because more proinflammatory cytokines are produced.

IL-6 may have proinflammatory or anti-inflammatory functions. The anti-inflammatory functions of IL-6 are caused by the suppression of IL-1 and TNF-α, the induction of glucocorticoid release, and the induction of natural antagonists of IL-1 (IL-1 receptor antagonist) and TNF (soluble TNFR p55) (Tilg et al). IL-6 is elevated in cases with inflamed pulp (Barkhordar et al, Zehnder et al 2003, Elsalhy et al).

As noted previously, IL-6 production is stimulated by IL-17. It may be involved in osteogenic (mineralizing) but not neurogenic differentiation of DPSCs (Park et al 2019). Together with $PGE_2$, TNF-α, and IFN-γ, IL-6 levels dropped after the administration of ibuprofen in human patients (Nguyen et al), showing the interrelationship of the production of these inflammatory mediators (Rankin et al).

IL-6 causes the formation of acute phase proteins in the liver such as fibrinogen and C-reactive protein (CRP). CRP functions as an opsonin by binding to C1q or Fcγ receptors or by activating the complement classical pathway. It has been shown that the dental pulp with irreversible pulpitis has significantly elevated CRP compared to uninflamed pulp, a finding that did not correlate with the patients' serum CRP (Proctor et al).

Another cytokine that is considered anti-inflammatory as well as an inducer of ECM production is TGF-β1. This cytokine is a member of the TGF-β superfamily, which includes activins and BMPs. However, TGF-β1 was shown to play a fundamental role not only in the formation of enamel and dentin matrix but also in the prevention of spontaneous pulpal inflammation (D'Souza et al). In that study, mice deficient in TGF-β1 (but treated with corticosteroids to keep them alive) developed very thin enamel and dentin with significant attrition and spontaneous pulpitis and pulp necrosis. Crossing this species with the RAG-2 mouse that is deficient in T- and B-cell immunity resulted in a breed that showed the same hard tissue defects but had normal pulp structure. The results indicate that TGF-β1 stops spontaneous inflammation mediated by the adaptive immune response. TGF-β1 is significantly increased in inflamed pulp but only in the odontoblastic/subodontoblastic layer, rather than the stromal pulp tissue (Piattelli et al). Interestingly, in vitro application of TGF-β1 to pulp tissue or odontoblasts upregulated proinflammatory cytokines and some anti-inflammatory cytokines, mostly IL-6, -7, and -8 (Paakkonen et al). Teeth with an immature apex have increased expression of TGF-β1 compared with mature teeth, which expressed more VEGF-A (Gomez-Sosa et al). TGF-β1 is sequestered in dentin (Smith et al), and when released from dentin by ethylenediaminetetraacetic acid (EDTA), it enhances DPSC growth and attachment to dentin (Sadaghiani et al).

Interestingly, normal dental pulp from patients with type 2 diabetes mellitus was recently shown to contain greater expression of inflammatory cytokines IL-1β, IL-6, IL-17, TNF-α, TLR2, and TLR4 compared to pulp from healthy controls without diabetes (Alsamahi et al).

**Toll-like receptors.** In addition to CD14, the effects of bacterial cell wall products are mediated by pattern-recognition

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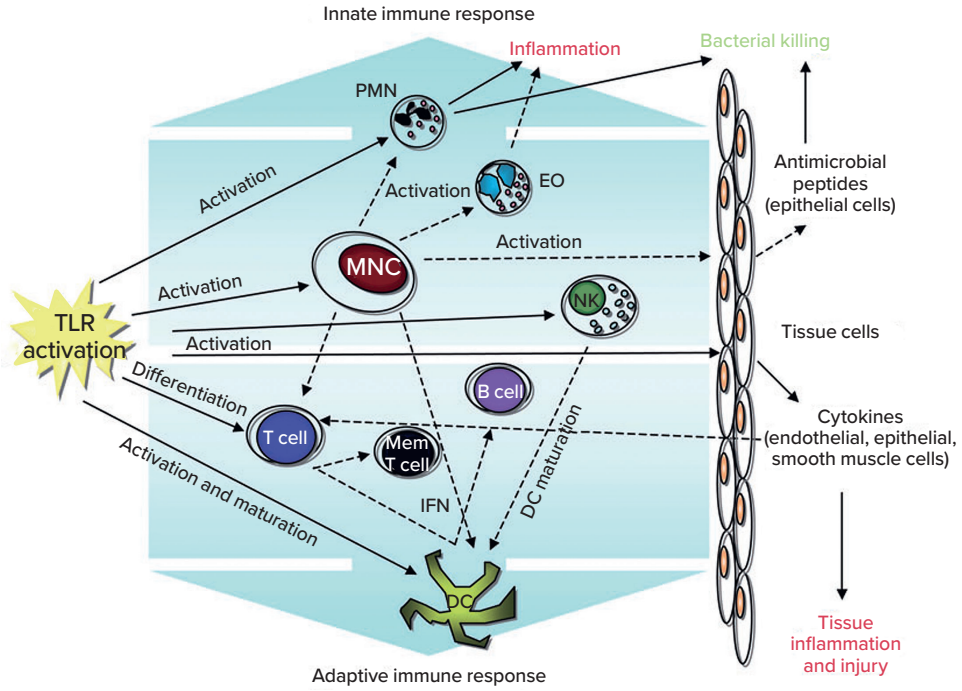
**TABLE 7-2** Number, location, and specificity of TLRs

| Organism/structure | TLR location | TLR designation |
|---|---|---|
| Bacterial lipopeptides | Cell surface | TLR1:TLR2 and TLR2:TLR6* |
| Bacterial peptidoglycan | Cell surface | TLR2 |
| Bacterial lipopolysaccharide | Cell surface | TLR4 |
| Bacterial flagellin | Cell surface | TLR5 |
| Viral dsRNA | Endosomes | TLR3 |
| Viral ssRNA | Endosomes | TLR7 and TLR8 |
| Viral, bacterial, and fungal DNA | Endosomes | TLR9 |
| Unclear function | Cell surface | TLR10 |

ds = double strand; ss = single strand. *Colon indicates a dimer. (Based on Abbas et al.)

molecules collectively known as *toll-like receptors* (TLRs) (Table 7-2). The identification of TLRs has extended our understanding of the mechanisms by which the innate immune system recognizes nonself and the important role played by TLRs in the detection of invading pathogens. Innate immunity has traditionally been considered a nonspecific process (compared to adaptive immunity) that is mediated via the engulfment and lysis of microbial pathogens by phagocytic cells such as macrophages and neutrophils and involves no complex protein-protein interactions. However, given the diversity of TLR functions, innate immunity is now recognized as a highly complex process in line with adaptive immunity. TLRs also play a crucial role in linking innate and adaptive immunity through action on T cells, particularly via interactions on dendritic cells (Fig 7-16).




◀ **FIG 7-16** Immune pathways activated by TLR signaling. TLR signaling activates several apical pathways that result in the stimulation of both the innate and adaptive immune responses. Mononuclear cells (MNCs) serve to amplify TLR activation by the production of cytokines and growth factors. These mediators facilitate communication between different cell types, coordinating the recruitment and activation of the immune response network. *Solid arrows* illustrate effects of direct TLR activation, and *dashed arrows* represent paracrine actions resulting from TLR activation of an intermediary cell. EO = eosinophils; DC = dendritic cell; Mem T cell = memory T cell. (Reprinted with permission from Parker et al.)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TLRs belong to a family of pattern-recognition receptors that recognize conserved parts of microbial components (ie, pathogen-associated molecular patterns [PAMPs]). The name "toll" stems from the recognition of the *Drosophila* Toll protein in fighting fungal infections.

Two important cell-surface TLRs are TLR4, for which the ligand is LPS (Hoshino et al), and TLR2, for which the ligands are PG and LTA (Schwandner et al). TLRs cause the translocation of several nuclear factors, including NFκB and AP-1 to the nucleus, followed by the transcription of the gene signal to produce the proinflammatory cytokines (Dinarello). LPS (endotoxin) was reported to be present in large amounts in carious dentin. It was also reported that superficial caries had more LPS than deep caries and that the LPS content correlated with the incidence of pain (Khabbaz et al). It has been reported that human trigeminal neurons that were TRPV1-positive nociceptors expressed TLR4 and CD14 (Wadachi and Hargreaves). In this study, it was shown that in inflamed pulp tissue, these receptors colocalized with N52, which is a marker of myelinated sensory neurons. TLR2 (Durand et al) and TLR4 (Jiang et al 2006) were also identified in odontoblasts.

It is of interest that these receptors are identified in such superficial locations in the pulp, where it is critical to detect bacteria and mount an immune response at an early stage. Not only do odontoblast-like cells express CD14 and TLR4, but the latter also appear to mediate the upregulation of the growth factor VEGF by these cells when they are stimulated by LPS (Botero et al). TLR2 was found to be strongly expressed and TLR4 faintly expressed in human dental pulp fibroblasts (Hirao et al). This suggests that fibroblasts also contribute to innate immunologic surveillance of the dental pulp, apart from their anabolic and catabolic roles in maintaining the structural integrity of the ECM. Upon activation of TLR2, human odontoblasts produced NO, especially NOS2, showing a functional role in antimicrobial defense (Farges et al 2015).

**NOD-like receptors and inflammasomes.** NOD-like receptors (NLRs) are a family of more than 20 cytosolic proteins, some of which recognize PAMPs and recruit other proteins to form signaling complexes that promote inflammation (Abbas et al). Inflammasomes are high–molecular weight complex proteins present in the cytosol of stimulated immune cells that mediate the activation of caspases (Broz and Dixit). Five main proteins have been described to assemble inflammasomes, including NOD (Broz and Dixit).

NOD2 is highly expressed in inflamed pulp (Keller et al 2011) and has the regenerative function of promoting odontoblastic differentiation (Lee et al 2014) via the NFκB/p65 signaling pathway (Xiao et al). NOD1 is involved in innate immune responses, such as chemokine production, by dental pulp cells (Hosokawa et al). Similarly, the NLRP3 inflammasome plays a crucial role in the detection of pathogens and in the progression of inflammatory and adaptive immune responses in odontoblasts and other dental pulp tissues (Song et al, Lv et al). The NLRP3 inflammasome and NOD2 are also thought to regulate the secretion and bioactivity of IL-1 family cytokines, TLR2, TLR4, chemokines, as well as human β-defensin 2 (Lee et al 2015, Al Natour et al 2023). The NLRP6-caspase 4 inflammasome was also significantly upregulated and activated in the dental pulp of patients with irreversible pulpitis through a mechanism that relies on the presence of type I IFN (Tian et al). Finally, caries-induced pulpitis was recently shown in an ex vivo model to be mediated by the NLRP3 inflammasome, which resulted in IL-1β production, suggesting that NLRP3 may be a therapeutic target in these cases (Al Natour et al 2023).

### Chemokines

Chemokines (chemotactic cytokines) are potent proinflammatory cytokines that are structurally homologous and act by mediating leukocyte movement and chemotaxis of inflammatory cells to the inflammatory site (Viola and Luster). They are also involved in angiogenesis. They may be classified based on functional criteria as follows: inflammatory cytokines are expressed by circulating leukocytes and other cells only upon activation, whereas homeostatic chemokines are constitutively expressed to promote constant monitoring of at-risk areas. A large number of inflammatory chemokines have been described (Table 7-3). Seventeen chemokine genes and 9 chemokine receptor genes were expressed in healthy human dental pulps (Farges et al 2009). Dental pulp cells exposed to endodontically relevant species of gram-positive or gram-negative bacteria expressed IL-8 and MCP-1 at both the protein and the mRNA levels (Jiang et al 1998). Human odontoblasts cultured in vitro are capable of expressing IL-8 at both the protein and mRNA levels (Levin et al). Patients with symptomatic or asymptomatic irreversible pulpitis had up to a 23-fold increase in IL-8 levels in the pulp (Huang et al 1999, Zehnder et al 2003, Wu et al) (see Fig 7-15). One study found that IL-6/IL-10 and IL-8/IL-10 ratios were significantly higher in symptomatic irreversible pulpitis compared with both caries-exposed and normal teeth (Elsalhy et al). In fact, pulpitis cases have significantly elevated IL-8, together with the neuropeptides NKA and SP as well as MMP-8, in the gingival crevicular fluid compared to normal teeth (Akbal Dincer et al). In addition, and as noted previously, SP was found to be a potent inducer of IL-8 and to a lesser degree MCP-1 (CCL2) in pulp cells and pulp tissue explants (Park et al 2004).

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**TABLE 7-3** Selected chemokines that have been identified in the dental pulp, their older names, and their major functions

| Chemokine | Original name | Major function |
|---|---|---|
| *CC chemokines* | | |
| CCL2 | MCP-1 | Mixed leukocyte recruitment |
| CCL3 | MIP-1α | |
| CCL4 | MIP-1β | T-cell, dendritic cell, monocyte, and NK recruitment |
| CCL5 | RANTES | Mixed leukocyte recruitment |
| CCL11 | Eotaxin | Eosinophil, basophil, and $T_h2$ recruitment |
| CCL17 | TARC | T-cell recruitment |
| CCL19 | MIP-β | T-cell and dendritic cell migration into parafollicular zones of lymph nodes |
| CCL20 | MIP-3α | $T_h17$ and dendritic cell recruitment |
| CCL21 | SLC | T-cell and dendritic cell migration into parafollicular zones of lymph nodes |
| CCL22 | MDC | NK and T-cell recruitment |
| *CXC chemokines* | | |
| CXCL1 | GRO-α | Neutrophil recruitment |
| CXCL2 | GRO-β | |
| CXCL8 | IL-8 | |
| CXCL9 | Mig | Effector T-cell recruitment |
| CXCL10 | IP-10 | |
| CXCL12 | SDF-1 | B-cell and T-cell migration into lymph nodes; plasma cell migration into bone marrow |

Based on Fouad and Huang, Abbas et al.

IL-8 (CXCL8) was significantly increased in cases of both symptomatic and asymptomatic irreversible pulpitis in one study that also investigated the changes in S100 proteins, which play a role in several innate immune responses and regenerative processes (Jungbluth et al). In this study, symptomatic irreversible pulpitis had elevated S100A8 and S100A14 levels, whereas asymptomatic irreversible pulpitis had elevated S100A14 and S100A16 levels.

IL-8 (CXCL8) is generally expressed in the pulp following bacterial recognition and triggering of the MYD88, NFκB, and MAPK pathways and in response to IL-17 production (He et al, Xiong et al). As the pulp degenerates and apical periodontitis ensues, IL-8 expression continues to increase (Rechenberg et al 2014).

Differentiated odontoblasts that were exposed to LTA in vitro expressed their own receptor, TLR2, as well as the chemokines CCL2 and CXCL10 (Durand et al). CXCL10 was also found to be upregulated in inflamed human pulp,


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primarily in macrophages, endothelial cells, and fibroblasts, whereas its receptor, CXCR3, was found on T cells (Adachi et al). In a model for pulpitis, CCL2, CXCL1, and CXCL2 were also elevated (Renard et al).

Chemokines not only induce cell locomotion but also influence angiogenesis. Among the chemokines expressed by odontoblasts in vitro, CCL2, CXCL2, and CXCL12 are proangiogenic, whereas CXCL4, CXCL10, and CXCL14 are angiostatic. Production of angiostatic chemokines in the healthy dental pulp in vivo might be involved in the maintenance of blood vessels beneath the odontoblastic layer. During inflammation caused by caries, the number of capillaries augments in the pulp under the lesion, and some of them penetrate the odontoblastic layer. The proangiogenic chemokine CXCL2 was strongly upregulated in LTA-stimulated odontoblasts and might contribute to the increased vascularization in inflammatory conditions by binding to CXCR2 receptors that are highly expressed on endothelial cells (Farges et al 2009). DPSCs conditioned by CGRP were recently shown to overexpress some proinflammatory cytokines and chemokines, including CXCL1 and CXCL8, which are both agonists of the receptor CXCR2 expressed in sensory neurons (Michot et al 2021). This suggests a role for DPSCs in mediating pain in pulpitis. Finally, CXCL12 (SDF-1) chemokine activity was the only one shown to increase in normal DPSCs and other dental pulp cells (Yan et al), and together with CXCR4, these chemokines played a critical role in odontoblastic differentiation (Kim et al 2014). By contrast, CCL11 (eotaxin) suppression was found to enhance stem cell–induced pulp regeneration (Hayashi et al).

## Other molecules of innate and adaptive immunity

### Class I and class II major histocompatibility complex molecules

The innate and adaptive immune responses work in synchrony to recognize and destroy foreign antigenic material. One of the fundamental mechanisms underlying this process is mediated by the major histocompatibility complex (MHC) class I and class II molecules. MHC class I is expressed on all nucleated cells, whereas MHC class II is expressed on a small group of cells collectively called *antigen presenting cells* (APCs). APCs include macrophages, dendritic cells, Langerhans cells, B cells, endothelial cells, and a few other cell types, although only MHC-1$^+$ dendritic cells and macrophages have been demonstrated in the dental pulp (Jontell et al 1998). MHC class I is recognized by CD8$^+$ or cytolytic T cells; the cells are referred to as *class I restricted*. This process is best known for viral infection of any nucleated cell, in which the viral antigens are recognized by the MHC class I–restricted CD8$^+$ cells. However, APCs process many other antigens, including bacterial antigens, and present them to MHC class II–restricted CD4$^+$ T helper cells. The processed antigens are recognized in the context of the MHC class II molecule on the APC and the T-cell receptor on the T cell. This mechanism is fundamental for the development of specific immunity to the presented antigen and the activation of T cells, macrophages, and B cells that follows. The expression of MHC molecules is mediated by cytokines, most notably IFN-γ.

Class II APCs were observed in normal human dental pulp, where they were localized to the odontoblastic layer and the central pulp (Jontell et al 1987). It was further shown that dendritic cells were much more important than macrophages in providing the antigen presentation signals to costimulate T cells in the rat pulp (Jontell et al 1994) and that these dendritic cells migrate to lymph nodes where they trigger adaptive immune responses (Bhingare et al). In the rat molar pulp, a dramatic increase in the number of class II APCs was seen following cavity preparation alone or with the application of *Porphyromonas gingivalis* LPS (Bergenholtz et al, Kamal et al). There was also a significant reduction in the number of these cells to baseline levels when the cavity preparations were immediately restored with a self-curing dental adhesive resin (Kamal et al). A possible functional relationship was demonstrated between pulpal APCs and sensory nerve fibers and their products. Sensory nerves were in close histologic proximity to the APCs, and in vitro experiments revealed that SP potentiated and CGRP suppressed the proliferation of stimulated T lymphocytes in the presence of APCs (Okiji et al 1997). An analysis of human teeth with superficial and deep cavity preparations, caries, and its treatment revealed that class II antigen presenting dendritic cells started accumulating in the odontoblastic area with deep cavities. While not detectable when the caries was restored, they reappeared 6 months after treatment (Yoshiba et al). This suggests that levels of these cells are closely related to the degree of external insult due to caries, cavity preparation, or microleakage after function. Taken together, these studies suggest that these immunocompetent cells play an important role in pulpal inflammation and interact with neuropeptides.

### Immunoglobulins

In addition to the specific cellular immunity factors discussed before, the dental pulp has long been shown to express humoral immunity under inflammatory conditions, where B cells differentiate to plasma cells that undergo clonal selection and produce the immunoglobulins IgG, IgA, IgE, and IgM (Pulver et al, Speer et al). Immunoglobulins serve in both recognition and effector functions. When antibodies are first expressed

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in a membrane-bound form on B cells, they recognize antigens in their environment. After their release, these antibodies mediate cytotoxic reactions by innate immune cells such as NK cells, opsonize bacterial and other antigens to enhance phagocytosis, and form antigen-antibody complexes that activate the classical pathway of the complement cascade. This leads to the secretion of anaphylatoxins and to the lysis of membranes of target cells as described previously, and in the case of IgE, they cause the degranulation of mast cells. The processes of opsonization and complement fixation take place via the Fc portion (non-antigen binding portion) of the antibody molecule, which is unique for each antibody isotype and binds to either Fc receptors on the surface of phagocytic cells for opsonization or to the C1q complement protein for complement fixation.

IgG1 was found to be the predominant immunoglobulin in normal dentin close to the pulp as well as in uninfected dentin under caries. When the pulp was sampled directly during pulpectomy procedures, significantly elevated levels of IgG, IgA, and IgM were reported in inflamed pulp compared with normal pulp (Nakanishi et al), indicating that the experimental technique may have influenced the findings in these studies. An additional protective function of immunoglobulins in the dentin-pulp complex was proposed with the finding that IgG, IgA, and IgM reduced fluid filtration through dentin in vitro (Hahn and Overton).

Immunoglobulins, especially IgG and IgM, may also mediate allogeneic responses during regenerative processes that involve grafting. In this regard, it was found that human dental pulp cells inhibit immunoglobulin production by B cells through the action of TGF-β (Kwack et al).

#### Defensins

Human α-defensins and β-defensins belong to many molecules that include LL-37, histatin, and other peptides with potent antimicrobial activities. These peptides have been shown to suppress gram-positive and gram-negative bacteria, fungi, and viruses. Some inflammatory cells, such as neutrophils, produce human α-defensins. Human β-defensins (hBD) were first described in relation to epithelial cells including oral mucosal and gingival keratinocytes. One study reported that odontoblasts express hBD-1 and -2, thus confirming the role that these cells play in the innate immune response of the dental pulp (Dommisch et al 2005). hBD-2 appears to be expressed in other human pulp cells through the TLR and NLRP3 inflammasome intermediates (Lee et al 2015). It was reported that the expression of hBD-1 and hBD-4, but not hBD-2 and hBD-3, was significantly increased in inflamed pulp compared with healthy pulp (Paris et al). As far as effectiveness against endodontic pathogens, hBD-3 peptide exhibited more antibacterial activity against mature multispecies biofilms containing *Actinomyces naeslundii*, *Lactobacillus salivarius*, *S mutans*, and *Enterococcus faecalis* or *E faecalis* alone in vitro than either saturated solution of calcium hydroxide or 2% chlorhexidine (Lee et al 2013a, Lee et al 2013b). hBD-3-C15 was also as effective as 2% chlorhexidine against *Candida albicans* (Yoo et al).

hBD-2 and TNF-α were significantly reduced in the dental pulp of smokers compared to nonsmokers, regardless of whether the pulp was normal or had symptomatic or asymptomatic irreversible pulpitis (Ghattas Ayoub et al). This may result in reduced pulp defense and account for why smokers have more root canal treatment than nonsmokers (Krall et al).

## Gene Polymorphism and Epigenetic and Systemic Factors in Pulpal Disease

### Gene polymorphism

Gene expression of various inflammatory mediators in the pulp is not uniform among all patients, just like for other tissues of the body. Single nucleotide polymorphism (SNP) in the genetic encoding of the proteins involved may lead to differences in the degree of expression of certain genes in different individuals. This may lead to increased susceptibility of some patients to severe inflammation that results in pulp degeneration, whereas others with seemingly similar clinical presentation may have a milder form of the disease (Fouad et al 2020).

Studies have examined gene polymorphism associated with the differences between patients with deep caries who had vital pulp and patients with pulp necrosis and the development of apical periodontitis. The underlying assumption in these studies was that variation in the expression of the studied genes may have led to the protection of the pulp in the group with vital pulp and the degeneration of the pulp in the group with apical periodontitis. Variation of 16 SNPs of MMPs or their inhibitors revealed a significant association for MMP-3 rs639752 and rs679620 genotypes with apical periodontitis (Menezes-Silva et al). Similarly, genetic alleles or polymorphism of IL-1β, IL-6, TNF, RANK, RANKL, and osteoprotegerin (OPG) were genotyped. Only an SNP in IL-1β (rs1143643) showed significant allelic and genotypic association with apical periodontitis (Dill et al). Another study of eight SNPs showed significant association with apical periodontitis of two SNPs in the heat shock protein family (HSPA1L and HSPA6) (Maheshwari et al). Heat shock proteins are induced by stress signals and play a


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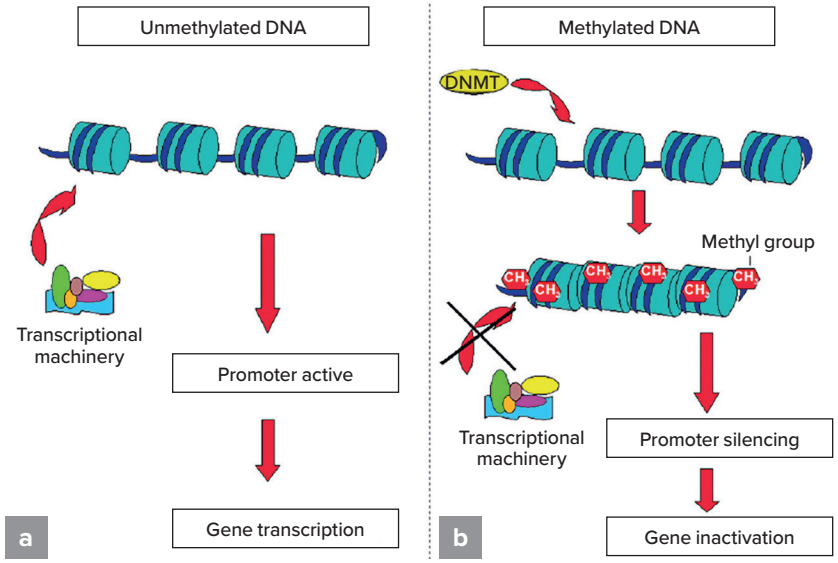


▶ **FIG 7-17** The effect of methylation in gene expression. *(a)* When the promoter region of DNA is unmethylated, there is an activation of gene expression. *(b)* DNA methylation occurs when methyl groups are added to cytosine nucleotides in specific areas of the gene by the enzyme DNA methyltransferase (DNMT). This chemical modification blocks the binding of transcription factors, inhibiting gene transcription. (Reproduced with permission from Cardoso et al 2010.)

role in protein synthesis, the initiation of innate immune responses, and the formation of proinflammatory cytokines. Yet other studies showed similar associations with an SNP for WNT3A and WNT5A genes (de Souza et al). In another area of research, pulpal sensitivity to electric stimuli was found to be significantly associated with female sex and the homozygous GG genotype rs165774 polymorphism for the gene that encodes for the enzyme catechol-O-methyltransferase (COMT) (Mladenovic et al). COMT inactivates dopamine, noradrenaline, and adrenaline—neurotransmitters involved in numerous physiologic processes, including pain modulation.

Taken together, these findings illustrate the complexity of the expression of inflammatory mediators in the pulp and the final effect that this may have on pulp survival.

## Epigenetic factors

In a similar fashion, there are many epigenetic variations that affect whether and to what degree a gene will be expressed. Epigenetic regulation does not cause changes in DNA sequence but is caused by changes in the chromatin structure or remodeling, histone variants, gene methylation, or the expression of various small non-encoding RNA sequences, commonly known as *microRNAs (miRNAs)* (Fouad et al 2020).

DNA methylation of cytosine-phosphate-guanine (CpG) in the *IFNγ* gene was shown to be associated with reduced expression of the gene in inflamed pulp compared to normal pulp (Cardoso et al) (Fig 7-17). In other studies, silencing of methyltransferase activity of enhancer of zeste homolog 2 (EZH2) in cultured human dental pulp cells attenuated the secretion of inflammatory cytokines (eg, IL-6, IL-8, IL-15, CCL2, and CXCL12) (Hui et al) and WNT/β-catenin–mediated dental pulp cell mineralization (Li et al 2018).

Epigenetic modification of DNA and DNA-associated histone proteins can control cell phenotype and regulate cell growth in stem cells. Nuclear enzymes including histone deacetylases and their inhibitors, as well as DNA-methyltransferase inhibitors, can affect DPSC differentiation (Duncan et al, Kearney et al). Furthermore, short-term activation of WNT signaling by WNT3A induced a genomic DNA demethylation and increased histone acetylation and histone methylation in DPSCs (Uribe-Etxebarria et al).

In a study of miRNA expression in normal and inflamed dental pulp, significant upregulation of three miRNAs—miR-150, miR-584, and miR-766—and significant downregulation of 33 others (of the total 335 miRNAs studied) was found (Zhong et al 2012). In another study, this group showed the expression of 12 viral miRNAs (vmiRs) in the pulp, of which 4 vmiRs (including those from herpesvirus and human cytomegalovirus) were differentially expressed in inflamed pulp. The differentially expressed vmiRs targeted key mediators involved in the detection of microbial ligands, chemotaxis, proteolysis, cytokines, and signal transduction molecules (Zhong et al 2017). Another miRNA, miR-223-3p, was implicated in the regulation of odontoblastic differentiation and in repair following pulpitis (Huang et al 2019). This miRNA was also found to be a negative regulator of the production

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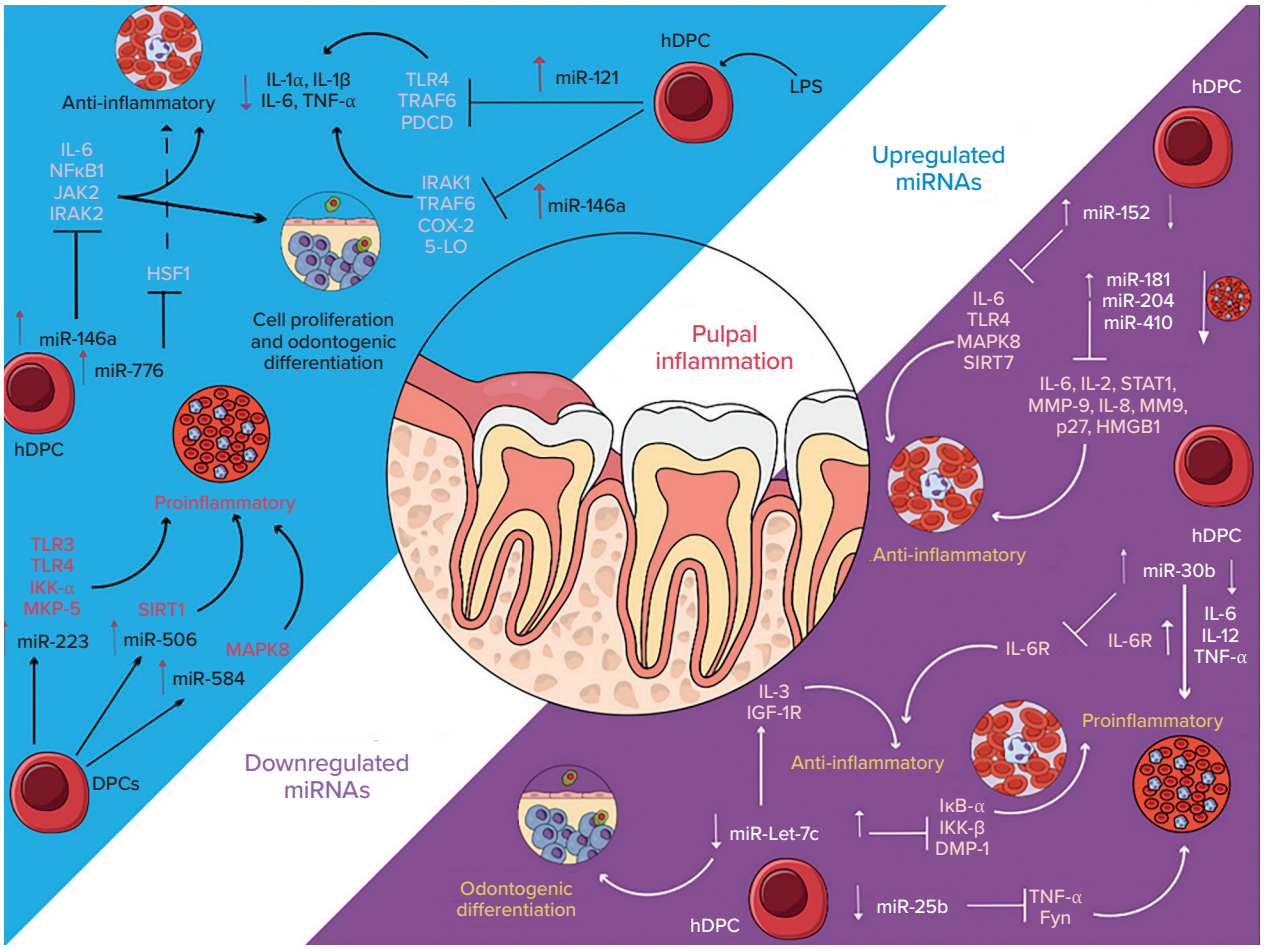
and secretion of proinflammatory cytokines mediated by the NLRP3/CASP1 inflammasome pathway by targeting NLRP3 (Wang et al). Other upregulated miRNAs in pulpal inflammation include miR-21, miR-146a, and miR-506 (Muñoz-Carrillo et al) (Fig 7-18).

A recent systematic review concluded that not only are miRNA expression profiles in DPSCs unique but that they regulate the levels of proteins crucial for promoting or inhibiting DPSC proliferation and differentiation and modulate inflammatory processes in dental pulp (Kulthanaamondhita et al).




▲ **FIG 7-18** Upregulated and downregulated miRNAs in pulpal inflammation. (Reprinted with permission from Muñoz-Carrillo et al.)

## Systemic factors

There is a growing body of evidence on the relationship between systemic disease and pulpal disease. Several chronic diseases mediate or enhance molecular mediators of pulpal inflammation, such as diabetes mellitus (Fouad et al 2002), sickle cell disease (Souza et al), and viral disease (Lopes et al, Li et al 2009). There are also significant changes that may occur in the pulp in response to chronic systemic medications that the patient is taking (Alghofaily and Fouad).

## Conclusion

The dental pulp produces a diverse number of molecular mediators that support development and homeostasis as well as both inflammatory and healing responses found in pulpitis. A great number of functions are ascribed to these mediators, and many of them may act in both propagation of disease and healing of diseased tissue. While complicated in their application, a better understanding of the functions of these factors may eventually lead to pharmacologic therapies that potentially reverse disease processes, mediating regeneration and the return of the dental pulp to its original form and function.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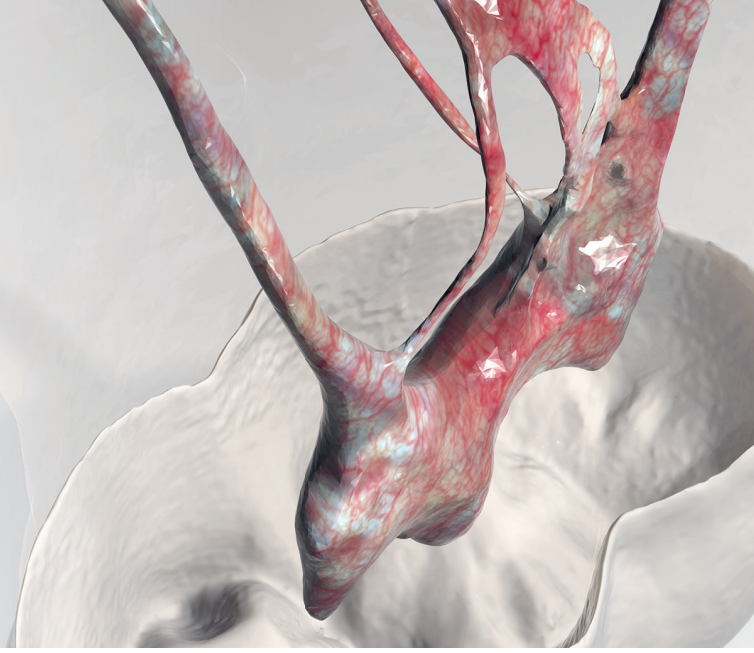
# 8

# INTERRELATIONSHIP OF THE PULP AND APICAL PATHOSIS

Renato Menezes Silva
Ariadne Letra
Gustavo Pompermaier Garlet



The dental pulp and periapical tissues are intimately related in terms of composition, resident cells, and effector molecules. As microorganisms from dental caries get closer to the dental pulp, odontoblasts, fibroblasts, immune cells, stem cells, and vascular and neural networks contribute with a variety of distinct defense mechanisms that are critical for tissue homeostasis. If left untreated, however, dental caries lesions advance into the pulp, leading to pulp necrosis and subsequent infection of the root canal system (Galler et al). The presence of bacteria and their byproducts within the infected root canal system will trigger the host local immune response, leading to inflammation of the periapical tissues and ultimately bone resorption with establishment of a periapical lesion, or apical periodontitis (Pitts et al, Stashenko et al 1992, Stashenko et al 1998). Notably, such pulpal and periapical inflammatory responses involve the participation of an extensive array of immune cells and molecular mediators contributing to complex immunologic mechanisms, some of which may act primarily to protect the dental pulp and periapical tissues while others mediate tissue destruction (Stashenko et al 1998, Bergenholtz 1990).

This chapter explores the interactions between the host immune system and pulpal and periapical tissues during dental pulp inflammation and the development and progression of apical periodontitis. Factors that can interfere with or promote tissue repair, wound healing, and regeneration are also discussed.

## Pulpal and Periapical Immune Responses

### Dental pulp inflammation

The dental pulp is a unique, specialized, loose mesenchymal connective tissue responsible for providing nutrition, oxygen supply, and innervation to the tooth (Zanini et al, Yu and Abbott). Similar to other body tissues, the dental pulp is well equipped to mount an immune-inflammatory response against pathogens and to stimulate healing and repair (Zanini et al). External insults to the dental pulp, whether from microorganisms (eg, dental caries, periodontal disease) or chemical, mechanical, or thermal factors, are able to induce a local inflammatory reaction in the pulp that is characterized by blood vessel dilation and a decrease in blood flow resistance (Tjäderhane). During acute inflammatory responses, vasodilation causes an increase in intravascular pressure and capillary blood flow that is critical for the delivery of immune system cells to the site of infection, eliminating bacterial toxins and metabolic waste products from the area (Takahashi).

Dental caries is one of the most prevalent chronic diseases worldwide and the most frequent cause of pulpal inflammation (Selwitz et al). Oral bacteria and their byproducts, such as lipoteichoic acid (LTA), can penetrate enamel and dentinal tubules, triggering inflammatory responses in the dental pulp (Farges et al 2015). Immune

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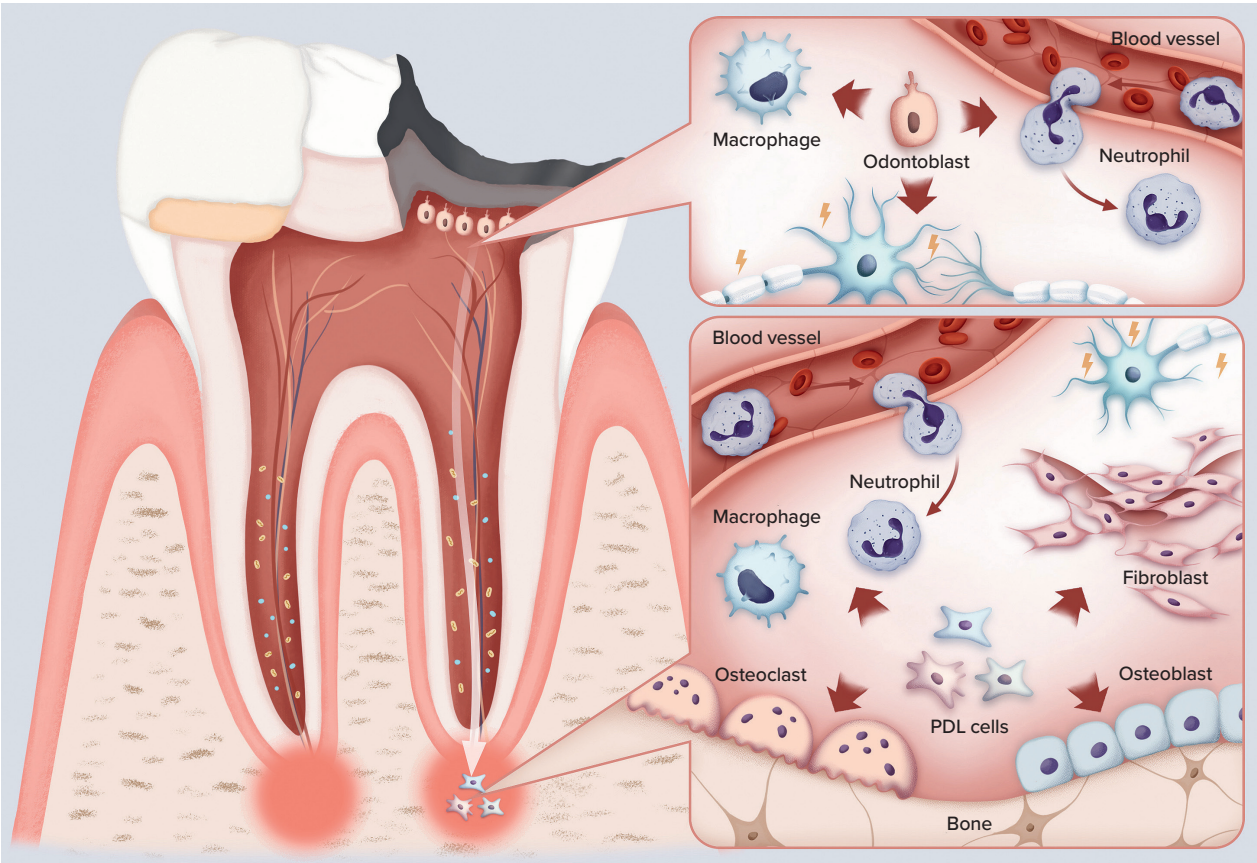
and inflammatory reactions may even be observed in the dental pulp before the caries lesion reaches the dentin (superficial caries) (Brannström and Lind, Bergenholtz 1981, Galler et al). If left untreated, caries lesions can cause dental pulp and root canal infection followed by the continuous flow of microorganisms and their metabolites (lipopolysaccharide [LPS] and LTA) to the periapical tissues, which will induce the mobilization of host response mechanisms and the development of apical periodontitis (Marton and Kiss 2000).

## Apical periodontitis

Apical periodontitis is a consequence of pulp necrosis and persistent polymicrobial infection within the root canal system. It is characterized radiographically as a periapical radiolucency indicating the resulting bony destruction and is usually diagnosed histologically as an apical granuloma or radicular cyst (Nair 2004, Kakehashi et al, Sundqvist, Zanini et al, Marton and Kiss 2014). It has been suggested that apical periodontitis is a host local immune/defense response to the infected root canal system and may prevent the spread of bacterial infections from the root canal to the surrounding tissues (Teixeira-Salum et al, Galler et al). However, the host defenses that act to eliminate the invading microorganisms may also result in tissue damage (Marton and Kiss 2014).

Approximately half of the adult population worldwide has at least one tooth with apical periodontitis (Tiburcio-Machado et al). Apical periodontitis has heterogeneous etiology, where the presence of specific bacterial taxa results in similar clinical outcomes (Roças and Siqueira). Factors that lead to persistent apical periodontitis include inadequate cleaning and shaping procedures, poor access, missed canals, and defective temporary or permanent restorations (Nair 2006) (Fig 8-1).




▲ **FIG 8-1** Schematic representation of the interrelationship of the dental pulp and apical pathosis. Odontoblasts comprise the first barrier against insults to the dental pulp and, along with other resident cells and vascular and neural networks, attract immune cells to the site of injury, initiating an immune-inflammatory response in an attempt to contain the tissue damage and promote repair. Similarly, in the periapical tissues, periodontal ligament (PDL) cells sense the dental pulp inflammation and mount a defense mechanism. As pulpal infection and inflammation progress, the dental pulp tissue may become necrotic, and apical pathosis is likely to develop.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## Resident and Immune Cells

The response of the dental pulp to caries, as well as the response of periapical tissues to root canal infection, may involve both innate and adaptive immune responses (Table 8-1), with numerous cells and molecular mediators working in a concerted effort to contain the microbial insult (Hahn and Liewehr). Odontoblasts are highly specialized cells and are the first line of defense against insults to the dental pulp. They are organized as a densely packed cell layer located at the periphery of the pulp and exert important functions as barrier cells. Odontoblasts are also able to recognize pathogens early in the process and secrete antibacterial compounds and chemokines, initiating the immune response and alerting and recruiting other key cellular players of the host defense (Galler et al, Veerayutthwilai et al, Hahn and Liewehr). Interactions between odontoblasts and pulpal dendritic cells (DCs) result in the expression of cytokines and prostaglandins and sequential recruitment of neutrophils, macrophages, and T and B cells. Odontoblasts are also involved in the production of vascular endothelial growth factor (VEGF), contributing to vascular permeability and new blood vessel formation in the dental pulp (Telles et al). As caries progresses into the dentin and the cellular infiltrate intensifies, odontoblasts signal to recruit additional immature DCs as well as pulpal fibroblasts, endothelial cells, and stem cells present in the perivascular niche, and then these odontoblasts die (Durand et al).

Fibroblasts are the most abundant cell type in the pulp tissue and have important roles during tissue repair and regeneration. They are located underneath the odontoblast layer and are also in contact with pathogens entering the dental pulp. Fibroblasts participate in the innate immune response by secreting component system molecules (cytokines and chemokines), expressing pattern-recognition receptors, secreting angiogenic growth factors, and regulating the neuronal plasticity process (Alvarez-Vasquez and Castaneda-Alvarado, Staquet et al).

While DCs try to phagocytize foreign antigens, a gradual accumulation of T helper ($T_h$) cells, cytotoxic T ($T_c$) cells, T regulatory ($T_{reg}$) cells, and B cells is observed. At later stages, antibody-producing plasma cells are also observed. Polymorphonuclear neutrophils (PMNs), monocytes, and natural killer (NK) cells continue to reside at the site of injury (Pulver et al, Farges et al 2015).

Monocytes, PMNs, and macrophages are also key cellular players in the innate immune system of the dental pulp and periapical tissues. PMNs are the predominant leukocyte cell population and among the first cells recruited to an inflammatory site; they are therefore considered major players during acute inflammation and are known as the "first line of defenders" (Castanheira and Kubes, Kolaczkowska and Kubes). Evidence suggests that PMNs are also involved in chronic inflammatory conditions and adaptive immune responses (Kolaczkowska and Kubes). DCs, macrophages, fibroblasts, T and B lymphocytes, and NK cells are present in the inflamed pulp tissue and are known to express several proinflammatory mediators important in the regulation of the immune responses of the dental pulp (Jontell et al). A healthy pulp tissue is also equipped with immune cells, with a majority of granulocytes/neutrophils ($CD16^+$, $CD15^+$, $CD14^-$) followed by $CD3^+$ T lymphocytes, $CD14^+$ monocytes, and DCs, together with minor subpopulations of NK cells, B cells, and $T_{reg}$ cells (Gaudin et al).

In apical periodontitis, periodontal ligament (PDL) cells are usually the first cells to interact with microorganisms and their byproducts, followed by an influx of leukocytes (Marton and Kiss 2014). PDL cells are known to express low levels of chemokines, which in turn provide a weak stimulus for recruiting and activating leukocytes. PMNs, T and B lymphocytes, plasma cells, DCs, NK cells, macrophages, eosinophils, mast cells, and osteoclasts are also important cellular players in the development of apical periodontitis. The earliest periapical tissue response usually occurs within 1 to 3 days after pulp exposure and involves an influx of PMNs and monocytes (Kawashima et al, Akamine et al). Increased osteoclastogenesis is evident by the third day after pulp exposure and seems to be dependent on the innate immune response (Rittling et al)

**TABLE 8-1** Characteristics of innate and adaptive immunity

| Innate immunity | Adaptive immunity |
|---|---|
| Nonspecific | Specific |
| Rapid response | Slow response |
| Limited potency | High potency |
| Initial response to pathogens | Activated if pathogens persist despite the innate response |
| Leukocytes | B lymphocytes |
| Natural killer cells | T lymphocytes |
| Plasma proteins | Antibodies in the blood and other body fluids |

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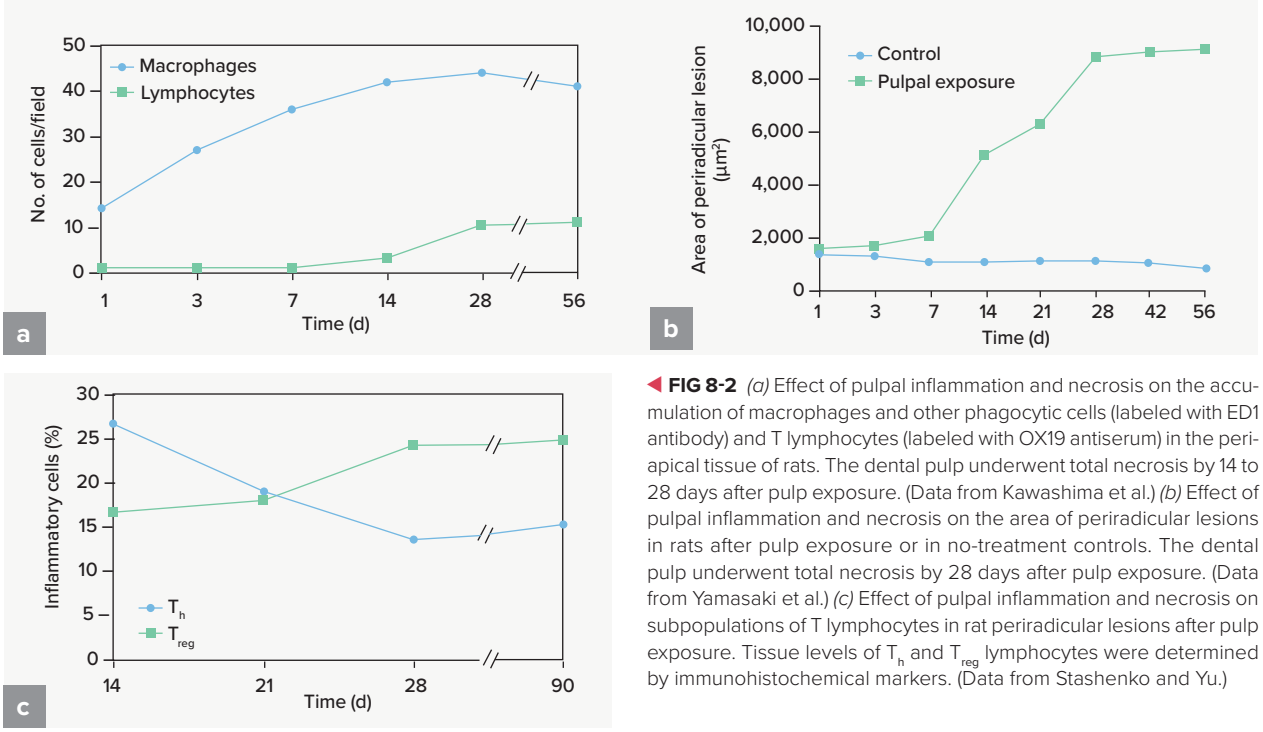


**FIG 8-2** *(a)* Effect of pulpal inflammation and necrosis on the accumulation of macrophages and other phagocytic cells (labeled with ED1 antibody) and T lymphocytes (labeled with OX19 antiserum) in the periapical tissue of rats. The dental pulp underwent total necrosis by 14 to 28 days after pulp exposure. (Data from Kawashima et al.) *(b)* Effect of pulpal inflammation and necrosis on the area of periradicular lesions in rats after pulp exposure or in no-treatment controls. The dental pulp underwent total necrosis by 28 days after pulp exposure. (Data from Yamasaki et al.) *(c)* Effect of pulpal inflammation and necrosis on subpopulations of T lymphocytes in rat periradicular lesions after pulp exposure. Tissue levels of $T_h$ and $T_{reg}$ lymphocytes were determined by immunohistochemical markers. (Data from Stashenko and Yu.)

(Fig 8-2 and Box 8-1). Similar to other leukocytes, neutrophils may present different phenotypes (Kolaczkowska and Kubes, Hirschfeld) and may be involved in later stages of lesion development. Neutrophil extracellular traps (NETs) have recently been suggested as a critical innate immune mechanism in infected dental pulp and periodontal tissues. They comprise a network of extracellular strings of DNA that bind to pathogens, limiting the dissemination of bacteria within the tissues. NETs also promote the attraction of macrophages, therefore facilitating removal of cellular debris (Holder et al, Moonen et al). Upregulation of SERPINB1, a potent inhibitor of neutrophil serine proteases (elastase and cathepsin G), in apical periodontitis tissues suggests a crucial role for this protein against neutrophil-induced periapical tissue damage (Cavalla et al 2017).

B lymphocytes, also called *B cells*, are effectors of the host adaptive immune response, with several immunologic functions including antibody production. Antibodies bind to pathogens and their toxins to neutralize them. Evidence has shown that B cells express interleukin (IL) 10 and are able to suppress immune responses (Mauri); they are sometimes called *B regulatory ($B_{reg}$) cells*. B cells work by internalizing antigens using an immunoglobulin receptor (ie, B-cell receptor) on the cell membrane. Once internalized, the antigen is degraded into peptides and attached to class II molecules of the major histocompatibility complex (MHC). The processed antigen is then transported to the B-cell membrane for presentation to T helper cells ($CD4^+$). B cells may also control and select antigens to be presented to T cells, particularly when the antigen concentration is low (Batista and Neuberger). Because B lymphocytes are rarely encountered in healthy human pulp (Gaudin et al), their role in pulpal inflammation is still unclear. Nevertheless, B lymphocytes have been observed in small numbers in cases of reversible pulpitis and in large numbers in cases of irreversible pulpitis. While their primary function is to produce antibodies, these cells can also function as antigen presenting cells, modulate DC functions, and produce cytokines (Bayry et al, Gaudin et al).

T cells are white blood cells that coordinate multiple aspects of adaptive immunity. More than 90% of lymphocytes in the healthy pulp tissue are T lymphocytes. A small number of T cells can be found in the normal pulp, primarily in blood vessels, most likely representing an immunosurveillance function (Jontell et al). There are three types of T cells: cytotoxic ($T_c$), helper ($T_h$), and regulatory ($T_{reg}$) (Kumar et al 2018). $CD4^+$ $T_h$ cells recognize exogenous antigens presented in the context of MHC class II molecules. $T_h$ cells produce cytokines that mediate inflammation and regulate other immune cells and are classified according to


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**BOX 8-1 Functions mediated by cells that infiltrate periapical lesions**

- **Neutrophils:** Phagocytosis (bacterial killing); cytokine production (IL-1, TNF-α)
- **Monocytes:** Phagocytosis (bacterial killing); immune induction; cytokine production (IL-1, TNF-α, IL-6, IL-10, IL-12); PGE production
- **Dendritic cells:** Antigen presentation; T-cell activation; cytokine production (IL-12, IFN-α)
- **Eosinophils:** Histamine release; immediate-type (anaphylactic) hypersensitivity
- **B cells:** Differentiation to plasma cells; low-level antibody production
- **Plasma cells:** Large-scale antibody production
  - IgM: Complement-mediated lysis; chemotaxis stimulated by C3a, C5a
  - IgG: Opsonization; immune complex formation; complement-mediated lysis; chemotaxis
  - IgA: Adhesion inhibition
- **T cells**
  - $CD4^+$
    - $T_h1$: Delayed-type hypersensitivity; IFN-γ, IL-12, TNF-α
    - $T_h2$: "Help" for antibody production; anti-inflammatory modulation (IL-4, IL-5, IL-6, IL-10, IL-13)
    - $T_h17$: Phagocytosis enhancement; proinflammatory modulation; IL-17, IL-22
    - $T_{reg}$: Regulation of T-cell proliferation; inflammation control; TGF-β, IL-10
  - $CD8^+$
    - Cytotoxic lymphocytes ($T_c$): Cytotoxicity; suppression

their cytokine expression and immune regulatory functions as $T_h1$, $T_h2$, $T_h17$, and T follicular helper ($T_{fh}$) cell subsets (Dong). $T_h1$ cytokines (interferon-γ [IFN-γ], IL-12) are associated with periapical bone destruction and the development and progression of apical periodontitis, while $T_h2$ antagonists (IL-4, IL-10, and IL-33) have been reported to attenuate tissue damage (Graves et al, Araujo-Pires et al 2014b). $T_h17$ cells mainly produce IL-17, but they can also produce other cytokines with osteoclastogenic properties, such as IL-6 and IL-23, reinforcing the potential destructive role of the $T_h17$ subset in apical periodontitis (Araujo-Pires et al 2014b, Takayanagi). $T_h9$ and IL-9 as well as $T_h22$ and IL-22 cytokines are also expected to contribute to apical periodontitis stability (Aranha et al). Other pleiotropic cytokines, such as IL-21 (a product of $T_h17$ or $T_{fh}$ cells), can also impact the overall immunoregulatory response and bone resorption process (Liu and King). $CD8^+$ $T_c$ cells recognize endogenous antigens presented in the context of MHC class I molecules and kill target cells by inducing apoptosis.

$T_{reg}$ cells act to suppress immune response, maintaining homeostasis and self-tolerance. $T_{reg}$ cells ($FOXP3^+$ $CD4^+$ subset) present suppressive effects on inflammatory bone destruction, which is thought to be mediated by cytokines such as transforming growth factor β (TGF-β) and IL-10 (Belkaid and Tarbell, Glowacki et al, Araujo-Pires et al 2014b, Campos et al).

Interestingly, interactions between $T_h17$ and $T_{reg}$ cells have been reported to influence the outcome of apical periodontitis (Fukada et al, Araujo-Pires et al 2014b). $T_h17$ cells promote osteoclast differentiation stimulated by receptor activator of nuclear factor κB ligand (RANKL) expression, while $T_{reg}$ cells try to inhibit this process by secreting TGF-β and IL-10 (Wei et al, Luo et al).

More recently, trigeminal neurons have also been reported to control the interaction between immune cells and bone cells, contributing to the development of apical periodontitis (Austah et al). Nociceptors were shown to regulate the early stages of apical periodontitis development by reducing the influx of macrophages and lymphocytes associated with decreased expression of IL-1β and IL-6, thereby promoting osteoblast differentiation and inhibiting osteoclast differentiation (Austah et al).

## M1/M2 macrophages

Macrophages are white blood cells with the capacity to phagocytose and kill microorganisms, clear dead cells and debris, and recruit other immune cells to the site of injury. Macrophages comprise an essential component of the innate immune system and a major immunocompetent cell type in the pulp and periapical tissues to fight against microorganism infection (Zhang et al). They may migrate to the site of infection during pulpitis and apical periodontitis or proliferate and differentiate in situ (Iwasaki et al, Marton and Kiss 2014). Macrophages also participate in the adaptive immune response as well as in tissue repair, regeneration, and remodeling processes (Bosurgi et al, Luo et al).

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Importantly, macrophages can present different phenotypes and functions; they can be classically activated (referred to as *M1 macrophages*) or alternatively activated (known as *M2 macrophages*) (Mills et al). M1 macrophages can be polarized by $T_h1$ cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor α (TNF-α), IFN-γ, and LPS. M1 macrophages express proinflammatory cytokines such as IL-1β, IL-6, IL-12, IL-23, and TNF-α. M2 macrophages are polarized by $T_h2$ cytokines IL-4 and IL-13 and express anti-inflammatory cytokines such as IL-10 and TGF-β (Zhang et al, Gordon and Martinez).

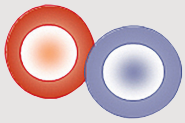
During dental pulp inflammation, fibroblasts stimulated by bacterial byproducts can induce macrophage differentiation into an M1 phenotype (proinflammatory), resulting in increased expression of TNF-α; meanwhile, injured fibroblasts signal to macrophages to assume an M2 phenotype (anti-inflammatory) with a significant increase in IL-10 secretion. M1 macrophages are usually present in the pulp zone underlying dental caries, whereas M2 macrophages are present in the peripheral inflammatory zone (Le Fournis et al).

It has been proposed that the development of apical periodontitis toward apical granulomas or radicular cysts may be influenced by macrophage polarization. Radicular cyst formation has been associated with increased M1 polarization, while apical granulomas present a higher degree of M2 polarization (Weber et al) (Table 8-2).

## Cellular response to microbial insults in the dental pulp

Successful defense against invading microorganisms involves rapid sensing through specialized recognition molecules. Microorganisms share similar signature molecules called *pathogen-associated molecular patterns (PAMPs)*, which facilitate their recognition by dental pulp and periapical tissue cells via pattern-recognition receptors. An important class of pattern-recognition receptors, the toll-like receptors (TLRs), are cell membrane receptors expressed in a range of resident and immune cells within the dental pulp and periapical tissues (eg, odontoblasts, fibroblasts, leukocytes, and mesenchymal stem cells); they are essential elements for triggering the innate immune response (Kumar et al 2011, Keller et al, Medzhitov, Cooper et al). TLRs can recognize PAMPs and additional bacterial surface components, including LPS, LTA, flagellin, peptidoglycans, and lipoproteins, as well as nucleic acid ligands from bacterial or viral pathogens (Medzhitov). Detection of bacterial components by TLRs (eg, TLRs 1 to 6 and 9) expressed on host cells triggers the innate immune response, alerting and recruiting additional cells from the

**TABLE 8-2 Roles of different cell types and their signature cytokines in the immune response**

| | PMN/M1 | M2 |  $T_h1/T_h17$ | $T_h2$ | $T_{reg}$ | B cell |
|---|---|---|---|---|---|---|
| Signature cytokine(s) | TNF, IL-1, IL-6 | IL-10, TGF-β | IFN-γ, IL-17 | IL-4 | IL-10, TGF-β | |
| Classic functions | Activation of inflammatory, immunologic, and vascular responses | Anti-inflammatory, pro-reparative | Cellular immune response, proinflammatory | Humoral immune response | Immune response suppression, immunologic tolerance | Antibody production, mediation of immunosuppression ($B_{reg}$) |
| Protective role | Infection control via antimicrobial response | Suppression of inflammatory response, induction of pro-reparative milieu | Activation of macrophage-mediated protection | Inhibition of $T_h1$ and $T_h17$ polarization, induction of $T_{reg}$ migration | Suppression of inflammatory response, induction of pro-reparative milieu | Infection control via antibody response, suppression of inflammatory response |
| Destructive role | Leukocyte chemotaxis, RANKL upregulation, matrix metalloproteinase upregulation | No evidence of destructive role | Proinflammatory loop, production of RANKL | Stimulation of B-cell response can increase RANKL production | No evidence of destructive role | Stimulation of B-cell response can increase RANKL production |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host defense mechanisms (Galler et al, Hahn and Liewehr, Veerayutthwilai et al). Upon TLR-pathogen binding, activation of the nuclear factor κB (NFκB) intracellular signaling pathway occurs, which is responsible for the regulation of the molecular inflammatory response (Medzhitov, Cooper et al). Binding of LPS to TLR4 or LTA to TLR2 induces the expression of chemokines, cytokines, and upregulation of T-cell costimulatory molecules (CD86, CD80, CD40), all of which play specific roles in the adaptive immune process (Hahn and Liewehr).

Odontoblasts constitutively express TLRs on their cell membrane. In shallow and moderate dentin caries, odontoblasts' response to gram-positive bacteria results in TLR2 upregulation, initiating the innate response and stimulating the production of proinflammatory cytokines IL-6 and CXCL8, as well as the immunosuppressive cytokine IL-10 (Farges et al 2011). Concurrently, this process also signals for the recruitment of immature antigen presenting DCs (Durand et al). Fibroblasts express TLR2, TLR3, and TLR4 and respond to LTA (through TLR2), viral dsRNA (through TLR3), and LPS (through TLR4) (Staquet et al).

In apical tissues, TLRs play an important role in the initial recognition of pathogens and their metabolites. TLR2 is expressed in lymphocytes and plasma cells, whereas TLR4 demonstrates proinflammatory properties when activated by LPS or endogenous damage-associated molecular patterns (Luo et al, Ciesielska et al).

## Molecular Mediators of Pulpal and Periapical Pathosis

### Cytokines and chemokines

One of the main consequences of TLR activation is the upregulation of cytokines and chemokines, resulting in the recruitment and activation of other immune cells (Turner et al, Farges et al 2015). Cytokines are secreted soluble proteins expressed by many cell types and have a major function in the modulation of inflammatory immune responses within the pulpal and periapical environment. Cytokines can influence the communication among immune and non-immune cells and act by binding to a specific cell surface receptor, generating a signaling cascade that affects cell function (Arango Duque and Descoteaux, Zhang and An). Cytokines are classified as proinflammatory and anti-inflammatory and may act on the cells that secrete them (autocrine action), on cells in the vicinity (paracrine action), or on distant cells (endocrine action) (Zhang and An).

The predominant cytokine producers are $T_h$ cells and macrophages (Zhang and An). During tissue infection, bacterial byproducts such as LPS and LTA can stimulate the inflamed pulp to express increased levels of cytokines, organizing a complex cellular immune response. Chronic inflammation and sustained cytokine release can result in dental pulp damage and a decreased capacity for tissue healing (Arora et al).

Chemokines are cytokines specialized in cell recruitment during inflammatory responses. They guide cellular migration in a process known as *chemotaxis* (Arango Duque and Descoteaux). Chemokines are responsible for the recruitment and subsequent activation of leukocytes, such as activated T cells, into inflamed tissues via chemokine receptors expressed on the cells (Adachi et al, Sallusto et al). Odontoblasts are important players in the production of chemokines when challenged by PAMPs (Farges et al 2015). They express CCL2, CXCL1, CXCL2, CXCL8, and CXCL10, attracting immune cells to the area beneath the caries (Durand et al, Staquet et al, Farges et al 2011). Expression of CCL2 by odontoblasts is considered a key factor for the recruitment of immature DCs during caries exposure (Durand et al, Staquet et al).

Increased levels of $T_h1$ type (CCR1, CCR5, CXCR3) and $T_h2$ type (CCR2 and CCR3) chemokine receptors were reported in periapical granulomas and periradicular cysts. Elevated expression of CCR3, CCR5, CXCR1, and CXCR3, in addition to chemokines RANTES, IP-10, and monocyte chemotactic factor (MCP)-1, in radicular cysts suggests a role for these molecules in the progression of periapical granulomas to cysts (Silva et al, Kabashima et al). Recent evidence also suggests that CXCL9 regulates macrophage migration and activation during the development of apical periodontitis and that tissue destruction may be contained by blocking CXCL9 (Hasegawa et al).

IL-1α- and TNF-α-positive cells are present as early as 2 days after pulp exposure in both the dental pulp and periapical region (Tani-Ishii et al). Marked levels of IL-1β, IL-6, IL-8, IL-10, TNF-α, and IFN-γ were observed in caries-exposed pulps and in irreversible pulpitis when compared to healthy pulp tissues (Elsalhy et al, Hirsch et al). IL-1β is a proinflammatory cytokine that is mainly produced by activated macrophages and regarded as a key regulator of the host response to microbial infection. IL-1β induces neutrophil influx, T- and B-cell activation, and antibody production (Zehnder et al, Elsalhy et al). IL-1β, IL-1α, and TNF-α can enhance bone resorption and inhibit bone formation (Nguyen et al). Increased levels of IL-1β and IL-1α have been correlated with severe inflammatory reactions as well as the progression of periapical granulomas (Yang et al).

IL-6 is expressed in the inflamed pulpal and periapical tissues (Barkhordar et al). It is mainly synthesized by macrophages but can also be expressed by other immune cells. IL-6 contributes to the differentiation and activation of $T_h2$, $T_h17$ and $T_{reg}$ cells (Farges et al 2015, Turner et al).

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In apical periodontitis, IL-6 levels have been reported to be directly proportional to the size of the periapical lesion and are highly expressed in symptomatic, epithelialized, and large lesions (Azuma et al).

IL-8 is expressed in inflamed dental pulp by macrophages, lymphocytes, fibroblasts, endothelial cells, and odontoblasts (Huang et al). IL-8/CXCL8 is considered a key complex associated with neutrophil recruitment and activation (Hahn and Liewehr). IL-8 was also detected in the cytoplasm of the Malassez epithelial cells, indicating its potential involvement in the development of apical periodontitis (Marton et al 2000).

IL-10 is an immunosuppressive cytokine produced by several cell types. IL-10 decreases the production of IL-6 and CXCL8, limiting tissue damage (Farges et al 2015, Li and Flavell). IL-10 is also capable of inhibiting $T_h1$ and $T_h2$ immune responses and promoting $T_{reg}$ differentiation (Saraiva and O'Garra). IL-10 expression has been reported in inflamed pulps and odontoblasts (Farges et al 2011, Farges et al 2015).

TNF-α is a proinflammatory cytokine produced by a variety of cell types, including macrophages, T cells, and NK cells. It plays a pivotal role in orchestrating the cytokine cascade (master regulator) in many inflammatory diseases (Parameswaran and Patial). For this reason, TNF-α has frequently been explored as a therapeutic target for several diseases (Parameswaran and Patial). TNF-α expression seems to be upregulated in irreversible pulpitis cases (Kokkas et al) and has been associated with posttreatment apical periodontitis (Dessaune Neto et al).

IFN-γ orchestrates many cellular effects such as upregulation of pathogen recognition, antigen processing and presentation, and leukocyte trafficking (Schroder et al). IFN-γ upregulates RANKL expression (Duka et al) but also promotes mesenchymal stem cell activity and survival (Wobma et al). Overall, the presence of TNF-α, IL-21, IL-17, and IFN-γ has been associated with active apical periodontitis lesions, whereas FOXP3, TGF-β, IL-10, IL-9, IL-4, and IL-22 have been associated with inactive or stable lesions (Araujo-Pires et al 2014b, Dessaune Neto et al).

## RANK/RANKL signaling pathway

RANK/RANKL signaling regulates osteoclast formation, activation, and survival. In contrast, osteoprotegerin (OPG), the decoy receptor for RANKL, is mainly produced by osteoblasts and protects against excessive bone resorption by binding to RANKL, thereby interfering with RANK/RANKL interactions (Boyce and Xing).

The inflammatory response, which is associated with root canal system infection, results in the upregulation of RANKL expression and the eventual loss of periapical bone (Francisconi et al). Evidence shows that osteolytic activity associated with the development of apical periodontitis may be related to a higher RANKL/OPG ratio; meanwhile, a low RANKL/OPG ratio is often observed in inactive or stable lesions (Menezes et al). Proinflammatory cytokines can also modulate RANKL/OPG expression. A good example is the capacity of IL-17 and IFN-γ to upregulate RANKL production, driving the progression of apical periodontitis, in addition to the pro-osteoclastogenic support provided by $T_h1$, $T_h17$, and B cells (Duka et al, Cavalla et al 2021).

RANKL can also play an important role in the regulation of the immune system by regulating the phenotype and function of DCs (Francisconi et al, Lin et al). RANKL inhibition may decrease $T_{reg}$ cell migration, resulting in low levels of $T_{reg}$-related cytokines IL-10 and TGF-β and thereby interfering with tissue repair and hemostasis and also contributing to tissue destruction and the progression of apical periodontitis (Francisconi et al, Luo et al). RANKL and OPG were reported to function as regulators of odontoclastic activity in the dental pulp of mice; OPG was dominantly expressed, suggesting a protective effect against odontoclastogenesis caused by dental pulp tissue damage (Nishida et al) (Fig 8-3).

## MMPs and TIMPs

Matrix metalloproteinases (MMPs) are a group of zinc-dependent enzymes strongly associated with levels of inflammation, tissue remodeling, and destruction (Elgezawi et al). They can collectively degrade all components of the extracellular matrix (collagen, proteoglycans, fibronectin, and laminin) and contribute to bone destruction (Everts et al, Kritikou et al). The function of MMPs is regulated by their inhibitors (ie, tissue inhibitors of metalloproteinases [TIMPs]), which try to contain the degenerative potential of MMPs (Bode et al).

MMPs play important roles during caries progression into dentin and the development of pulpitis, as well as apical periodontitis (Letra et al, Jain and Bahuguna, Elgezawi et al). They can be synthesized by odontoblasts, PMNs, macrophages, and plasma cells (Kritikou et al, Elgezawi et al). Increased release of active MMPs was observed in inflamed dental pulp when compared to healthy dental pulp (Kritikou et al), demonstrating their importance in tissue remodeling and destruction during pulpal inflammation and further apical periodontitis development.

The balance between MMPs and TIMPs has been suggested as an important factor in the pathogenesis and progression of apical periodontitis (Letra et al, Corotti et al). Increased expression of MMPs 2, 7, and 9 has been noted in the periapical tissues of patients with chronic


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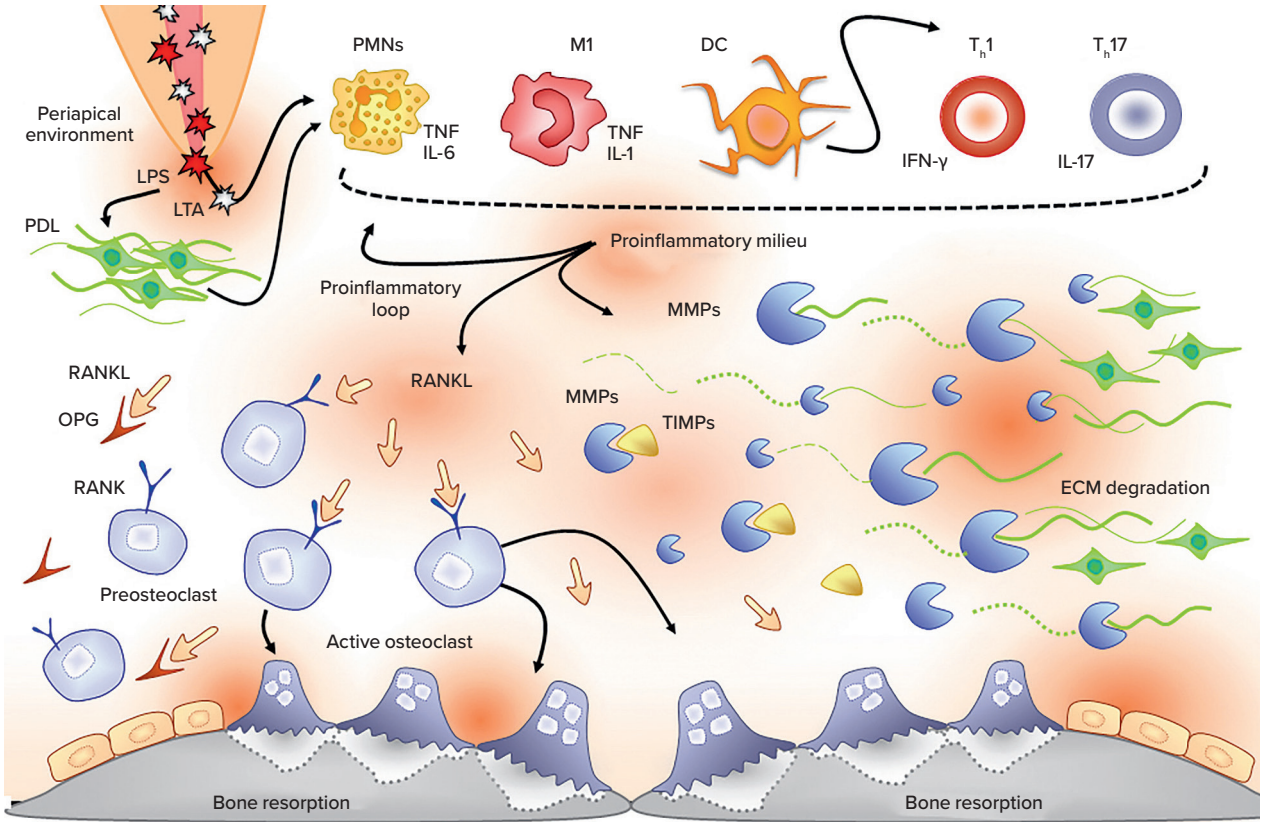


▲ **FIG 8-3** Schematic representation of the immunologic features of active/progressive periapical lesions. PDL cells are the initial cells exposed to microorganisms and their byproducts and trigger the release of inflammatory and chemotactic factors that initiate the local influx of leukocytes. PMNs are the first cells recruited to the inflammatory site during acute inflammation, followed by macrophages. These innate immune cells recognize the microbes' signature molecules (PAMPs) via pattern-recognition receptors and release proinflammatory mediators, such as TNF, IL-1, and IL-6. The subsequent steps of host response involve DCs, which respond to the microbial and inflammatory stimuli and migrate to draining local lymph nodes to activate the adaptive immune response. Once the adaptive response is initiated, polarized CD4 lymphocyte subsets, such as $T_h1$ and $T_h17$, can reach the inflamed periapical environment and produce its prototypic cytokines, namely IFN-γ and IL-17 (respectively), which in turn create a proinflammatory loop via stimulation of both M1 macrophages and PMNs. Noteworthy is that this sustained proinflammatory response triggers tissue destruction by disrupting the homeostatic balance that regulates soft tissue and mineralized tissue turnover. Osteoclast differentiation and activation is driven by the interaction of RANK with its ligand RANKL, an event regulated by OPG, a decoy receptor of RANKL that inhibits RANK/RANKL engagement. The overexpression of RANKL in inflammatory conditions results in an imbalance of RANKL/OPG levels, leading to excessive osteoclast activity and consequently to pathologic bone resorption. Similarly, the proinflammatory elements result in the upregulation of matrix metalloproteinases (MMPs), a group of zinc-dependent enzymes responsible for tissue remodeling, but without an equivalent increase in their tissue inhibitors (ie, TIMPs), this ultimately results in increased extracellular matrix (ECM) degradation. Collectively, RANKL/OPG and MMP/TIMP imbalances result in the pathologic tissue destruction characteristically associated with the development of apical periodontitis.

apical abscesses, whereas TIMP1 was increased in the tissues of patients with asymptomatic apical periodontitis (Letra et al).

## Wound Healing

The interrelationship of the infected pulp/root canal system and the development of apical pathosis is a critical aspect during wound healing. The development of precise diagnostic tests and treatment options to promote healing and maintain dental pulp vitality after pulp infection and inflammation is a topic that still requires attention. The same applies for apical healing after pulp necrosis and apical periodontitis development.

Elimination of microorganisms and prevention of pulp and root canal recontamination are prerequisites for successful treatment outcomes (Tjäderhane). Wound healing is a well-coordinated process that involves the activation of remodeling enzymes, extracellular matrix components, growth factors, cellular adhesion molecules, cytokines, and chemokines (Garlet et al). The healing

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process is thought to begin immediately after tissue injury, but prolonged injury and inflammation and additional systemic and local factors might disturb the natural course of healing, resulting in a chronic, nonhealing lesion (Yager and Nwomeh).

Interactions between resident, immune, and stem cells are important biologic events during tissue repair (Araujo-Pires et al 2014a). Mesenchymal stem cells play a fundamental role in tissue repair, as they may survive periapical inflammation and act as immunosuppressive agents. Mobilization and recruitment of mesenchymal stem cells to the wounded site may provide support to bone formation and neuronal cell differentiation (Liao et al, Marrelli et al, Araujo-Pires et al 2014a). Ultimately, the balance between pro- and anti-inflammatory mediators will determine the stable or progressive nature of pulpitis and apical periodontitis (Cavalla et al 2021).

Several genes are activated during healing events. *SERPINE1*, *TIMP1*, *COL1A1*, *COL5A1*, *VTN*, *CTGF*, *FGF7*, *TGFB1*, *TNF*, *CXCL11*, *ITGA4*, and *ITGA5* have been described as important factors in the wound healing cascade in apical periodontitis tissues (Garlet et al). Intriguingly, the same mediators that promote healing may participate in tissue destruction (Kondo and Ishida). Protective and destructive immunoreactions may take place simultaneously in the dental pulp and periapical tissues. A low-grade pulpal inflammation may promote regenerative mechanisms including angiogenic and stem cell processes (Cooper et al). Growth factors, neuropeptides, cytokines, and chemokines released from the host dentin matrix, pulpal and periapical cells, immune cells, and neurons will modulate defense and repair processes within the tissues (Cooper et al, Garlet et al).

## Genetic Polymorphisms, DNA Methylation, and miRNAs

The interplay between multiple immunoregulatory factors may determine the dynamics of apical periodontitis development. The extent and duration of the host response may play a major role in the determination of healing versus destructive processes, and a complex signaling network operates in the determination of lesion outcome (Garlet et al).

While genetic association and epigenetics cannot be directly linked to pulpal and periapical inflammation and tissue destruction, identifying new mechanisms that could help predict host susceptibility to develop pulpitis and apical periodontitis and also the individual's healing capacity to treatment would be of incredible value (Fouad et al). A single nucleotide polymorphism (SNP) is a DNA sequence variation characterized by substitution of a single nucleotide in the genome sequence. It is present in at least 1% of the human population and, depending on its position in the gene as well as the main function of the affected gene, may contribute to altered predisposition to or protection against certain diseases (Stankiewicz).

Genetic polymorphisms have a role in host susceptibility to several diseases, including pulpitis and apical periodontitis. SNPs in a number of genes, most of which belong to immune response–related pathways, have been reported in association with apical periodontitis predisposition (de Souza et al, Souza et al, Dill et al, Menezes-Silva et al, Petty et al). Specifically, genes belonging to the interleukin and MMP gene families that are involved in the signaling cascade and tissue degradation observed in apical periodontitis have been reported in association with apical periodontitis in multiple studies (Dill et al, Menezes-Silva et al, Trombone et al, Evrosimovska et al, Aminoshariae and Kulild, Petty et al). Importantly, the effects of genetic predisposition have been reported in support of potential associations between apical periodontitis and systemic diseases (Messing et al, Petty et al), although this is still an emerging area of study in the endodontics field.

Epigenetics is the study of genetic regulatory mechanisms that can repress gene expression, determining which proteins are transcribed without changing the DNA sequence. *DNA methylation* refers to epigenetic events in which additional methyl groups are added to the DNA structure. It occurs in a region in which a cytosine nucleotide is located next to a guanine nucleotide that is linked by a phosphate (also known as *CpG islands*). CpG islands are methylated by one of three enzymes called *DNA methyltransferases*. Inserting methyl groups changes the appearance and structure of DNA, modifying a gene's interactions with the machinery within a cell's nucleus that is needed for transcription (Robertson). Moreover, the effects of DNA methylation are often observed as a transcriptional genetic modulation; hypermethylation is often reported to result in a decrease in gene expression, while hypomethylation is usually related to transcriptional upregulation (Campos et al). FOXP3 DNA methylation levels were markedly increased in cases of apical periodontitis (Campos et al). FOXP3 expression governs the development and function of $T_{reg}$ cells, whose functions include inhibition of immune responses.

MicroRNAs (miRNAs) are a class of conserved noncoding RNAs that regulate gene expression predominantly at the posttranscriptional level. They are encoded in both intergenic and intragenic regions of the genome (Bartel 2004, Bartel 2009). Recent studies have explored the roles of miRNAs and their pro- and anti-inflammatory properties in the dental pulp and periapical tissues (Shen and Silva, Yue et al). Over 800 miRNAs were reported to be expressed in apical periodontitis tissues. Upregulated miRNAs were correlated with osteoclast differentiation


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and the TNF signaling pathway (Shen et al). Members from the miR-181 family were downregulated in apical periodontitis. Their primary target genes were IL-6, MMP-9, and TGF-β (Chan et al).

## Conclusion

Several studies have identified and elucidated the roles of many cellular and molecular players in caries and pulpal and periapical tissue inflammation, contributing to a better understanding of the disease mechanisms and interrelationship of the pulp and apical pathosis.

It is well accepted that pulpitis and apical periodontitis are complex diseases characterized by tightly coordinated immunologic mechanisms of host defense, some of which may act primarily to protect the dental pulp and periapical tissues while others mediate tissue destruction. In recent years, advances in research technologies have strengthened the evidence of host-mediated mechanisms contributing to apical periodontitis development, including the influence of host genetics and epigenetics on the regulatory factors that determine the dynamics of apical periodontitis development and healing. As studies in the field continue to progress, these findings will be of value for clinical translational research findings; molecules identified as critical players in the development and/or progression of apical periodontitis may be used as targets for precision dentistry approaches in addition to conventional endodontic treatment.

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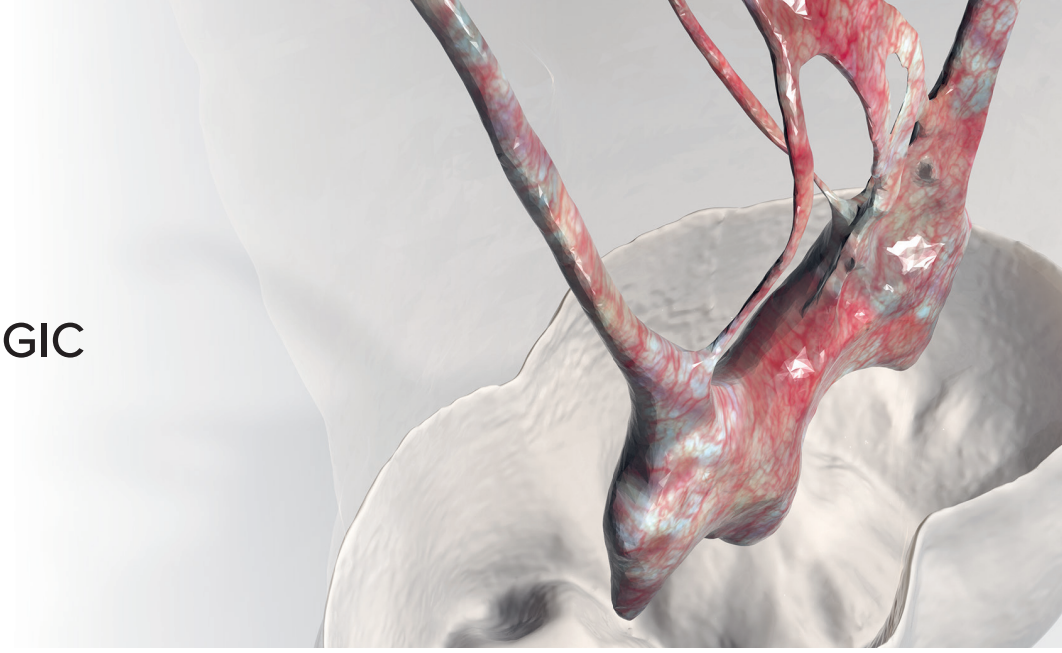
# 9

# PHARMACOLOGIC CONTROL OF DENTAL PAIN

Asma A. Khan



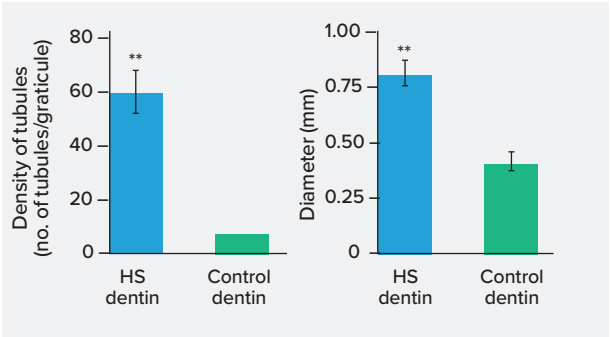
Odontalgia (toothache) is the most common chief complaint in patients seeking emergency dental care. It is the predominant sensation following activation of the sensory neurons that innervate the dental pulp and dentinal tubules. Although patients may report their pain as a "toothache," the skilled clinician understands that this single response may be an integration of pain originating from pulpal and periradicular nociceptors (eg, symptomatic apical periodontitis) in addition to central mechanisms of hyperalgesia. Moreover, in some cases, the noxious input may not even derive from odontogenic tissues; it may actually represent pain referred to the site from a distant tissue and integrated in the central nervous system (see chapter 15).

Given this complexity, it is perhaps not surprising that many patients view dentistry and pain as synonymous. Indeed, studies surveying more than 45,000 households in the United States indicate that odontalgia, or toothache, is the most common form of pain in the orofacial region, afflicting 12% of the study population (Lipton et al). This percentage corresponds to about 20 million people in the United States alone.

This chapter reviews studies on treatment of the two major forms of dental pain—dentinal sensitivity and inflammatory pulpal pain. Evidence supporting pharmacologic and nonpharmacologic treatment regimens for management of dental pain are reviewed in the context of clinical interventions.

## Management of Dentinal Hypersensitivity

Dentinal hypersensitivity, a relatively common finding in adult populations, is characterized by sharp pain arising from the stimulation of exposed dentin. Identified risk factors for dentinal hypersensitivity include erosion, abrasion, attrition, gingival recession, periodontal treatment, and anatomical defects (Cox, Bissada 1994, Addy 1990). Teeth with dentinal hypersensitivity demonstrate significantly greater numbers of patent dentinal tubules and a significantly greater mean diameter per dentinal tubule than control teeth (Absi et al) (Fig 9-1). Accordingly, interventions that reduce either fluid flow or the activity of the neurons that innervate dentinal tubules would be predicted to be effective in reducing dentinal hypersensitivity.




▲ **FIG 9-1** Comparison of the density of dentinal tubules and the mean diameter of dentinal tubules in teeth taken from 34 patients with hypersensitive (HS) dentin and 37 patients with normal dentin. Teeth were extracted and then examined under a scanning electron microscope. $^{**}P < .01$ versus control dentin. (Data from Absi et al.)

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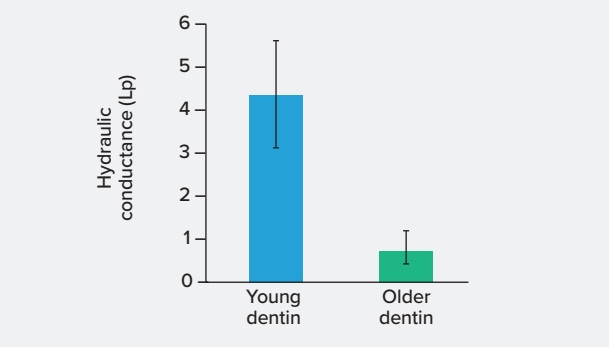
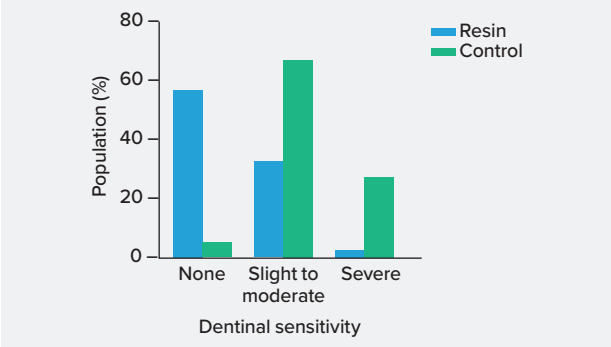



▲ **FIG 9-2** Hydraulic conductance of dentin discs taken from younger (22- to 27-year-old) and older (45- to 62-year-old) patients. Lp = liquid permeability. (Data from Tagami et al.)




▲ **FIG 9-3** Effect of applying an unfilled resin (Concise Enamel Bond) to exposed dentin in 51 pairs of contralateral premolars in an experimental clinical model of dentinal hypersensitivity. A consistent amount of dentin exposure was created on the premolars, which then were either covered with resin or served as a no-treatment control. Dentinal sensitivity was assessed by a short pulse of compressed air on the exposed dentin. (Data from Nordenvall et al.)

Several cross-sectional studies have reported on the clinical characteristics of dentinal hypersensitivity. The reported prevalence of dentinal hypersensitivity ranges from 3.8% (N = 3,593, United Kingdom) to 17% (N = 635, Brazil) to about 55% (N = 250, Ireland; N = 277, United Kingdom) (Rees, Irwin and McCusker, Gillam and Newman). The wide variation in the reported prevalence may be related to cultural or genetic factors or to experimental variations in methods of assessment or sampling. Indeed, several investigators have proposed standardized methods for assessing dentinal hypersensitivity and conducting randomized controlled clinical trials (Holland et al, Gillam and Newman).

Dentinal hypersensitivity has been reported to have a higher incidence in female patients (Addy 1990). The peak reported period for dentinal hypersensitivity is the third to fourth decades of life. Most studies report a decline in the prevalence of dentinal hypersensitivity in older patients, which may be related to reductions in dentinal tubule permeability (Tagami et al) (Fig 9-2).

The locations most often affected tend to be the cervical region of incisors and premolars, often on the side opposite the dominant hand. This latter finding is consistent with toothbrush abrasion as an etiologic factor. Dentinal pain is elicited by cold stimuli in up to 90% of patients, and mechanical (eg, toothbrushing) and chemical (eg, candy) stimuli can also induce pain (Rees, Irwin and McCusker, Gillam and Newman).

The predominant hypothesis for dentinal hypersensitivity has been Brännström's fluid flow hypothesis. It is now known that odontoblasts function as sensory cells that can transduce external stimuli. Recent data show that human odontoblasts express functional thermosensitive transient receptor potential channels (TRP), namely TRPV1, TRPA1, and TRPM8 (El Karim et al). TRPA1 and TRPM8 are cold-sensitive and are activated at temperatures below 17°C and 25°C, respectively. TRPV1 is activated by noxious heat. Odontoblasts also express other ion channels known to play a role in sensory reception, including voltage-gated sodium channels, mechanosensitive K channels, and L-type calcium channels (Allard et al 2000, Allard et al 2006, Magloire et al).

Several treatments have been evaluated for the management of dentinal hypersensitivity. These interventions generally focus on reducing the permeability of dentinal tubules or reducing the sensitivity of dentinal neurons.

## Interventions that reduce permeability of dentinal tubules

Because fluid flow is a major stimulus for activating nociceptors that innervate dentinal tubules, it is not surprising that interventions that reduce the permeability of dentinal tubules have been evaluated for reducing dentinal hypersensitivity. The application of resins to exposed dentinal tubules has been reported to reduce dentinal hypersensitivity of multiple etiologies at up to 12 months' follow-up (Gillam and Newman, Brännström et al, Felton et al).

One group of researchers developed an experimental model of dentinal sensitivity by producing uniform dentin exposures in premolars (prior to tooth extraction for an orthodontic indication) (Nordenvall et al). These investigators demonstrated that the application of Concise Enamel Bond (3M ESPE) to the exposed tubules produced about a twofold reduction in dentinal sensitivity compared with control teeth. Similar results were reported by the same group for actual clinical cases of dentinal hypersensitivity (Nordenvall et al) (Fig 9-3).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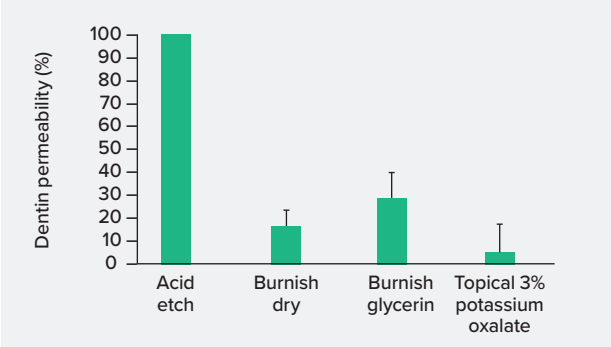
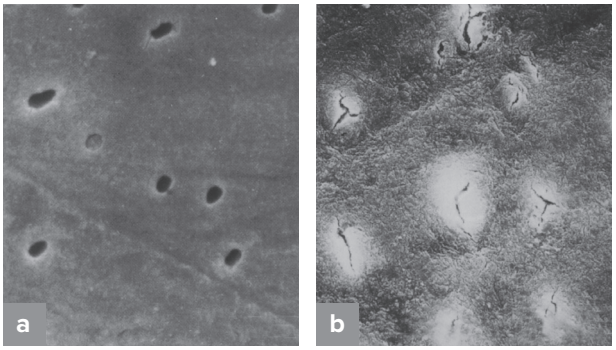



▲ **FIG 9-4** Effect of burnishing on in vitro dentin permeability. Dentin permeability after original acid etching is assigned a value of 100%. (Redrawn after Pashley et al.)




▲ **FIG 9-5** Scanning electron photomicrographs of the surface of human radicular dentin. *(a)* Hypersensitive dentin. *(b)* A tooth 6 months after application of a resin liner. Note the occluded tubules. The patient reported a lack of dentinal sensitivity after the application of resin. (Reprinted with permission from Yoshiyama et al.)

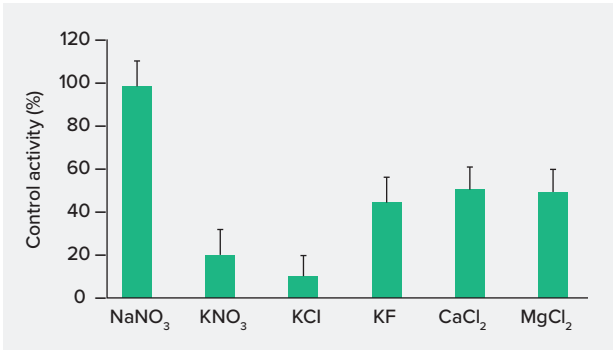
Other interventions that block fluid flow have also been reported to be effective in treating dentinal hypersensitivity. Examples include the application of materials such as Gluma Dentin Bond (Heraeus Kulzer), oxalate salts, isobutyl cyanoacrylate, and fluoride-releasing resins or varnishes; $CO_2$ lasers; the use of devices that burnish exposed dentin (Fig 9-4); and even coronally positioned mucogingival flaps (Felton et al, Pashley et al, Javid et al, Tavares et al). The dominant factors contributing to the efficacy of these interventions are the longevity of effective blockage of tubules and the limited potential for cytotoxicity. For example, patients who report continued efficacy of resins at 6 months after application have evidence of resin tags still blocking their dentinal tubules; patients who have recurrence of dentinal sensitivity have few or no resin tags remaining in their tubules at that point (Yoshiyama et al) (Fig 9-5).

However, many studies are simply before-and-after comparisons, and few studies directly compare efficacy and adverse effects among several active treatments. The lack of direct comparisons and systematic evaluations makes it difficult to determine which of the proposed treatment regimens offers the greatest efficacy and duration with the least potential for adverse effects. Thus, further research is required in this important therapeutic area.

## Interventions that reduce sensitivity of dentinal neurons

The second general method to reduce dentinal hypersensitivity enlists interventions that reduce neuronal responsiveness to dentinal stimuli. Both preclinical studies and clinical trials have been used to evaluate these agents.

The preclinical studies often employ multiunit intradental electrophysiologic recordings of fibers that innervate dentinal tubules. For example, some researchers have used an anesthetized feline model with multiunit recordings of dentinal neurons using a pretreatment design (Markowitz and Kim). In this design, desensitizing agents were applied to exposed dentinal tubules prior to the application of excitatory agents (eg, hypertonic sodium chloride). This model has been used to evaluate the efficacy of numerous compounds for reducing activation of dentinal neurons. In general, these studies indicate that *(1)* the application of potassium reduces neuronal activity regardless of the paired anion, *(2)* divalent cations are effective inhibitors of dentinal neurons, and *(3)* nitrate ($NO_3$) is not effective in altering neuronal activity (Markowitz and Kim) (Fig 9-6). However, it should be noted that the deep cavity preparations used in this model (ie, 20 to 50 mm of remaining




▲ **FIG 9-6** Effect of testing desensitizing agents on dentinal neuron activity in exposed dentin. Data are presented as percentages of neuronal activity compared with that of a control (ie, application of hypertonic [3.0 M] sodium chloride is defined as 100%). The test agents are sodium nitrate ($NaNO_3$), 1.10 M; potassium nitrate ($KNO_3$), 1.0 M; potassium chloride (KCl), 0.76 M; potassium fluoride (KF), 0.05 M; calcium chloride ($CaCl_2$), 0.76 M; and magnesium chloride ($MgCl_2$), 0.76 M. (Adapted from Markowitz and Kim.)

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dentin) might lead to an overestimation of the drug efficacy because diffusion through dentinal fluid has been minimized. Moreover, no clinical studies to date have demonstrated that the concentrations of the test agents achieved in these experimental models are similar to actual concentrations achieved during therapeutic use in human dentinal fluid.

Several major reviews have summarized the outcomes of clinical trials evaluating desensitizing agents (Orchardson and Gillam, Kanapka). As already mentioned, variations in the methodology or design of the clinical trials (ie, techniques for assessing hypersensitivity, study duration, data analysis, comparison to active controls) may lead to differences in study outcomes. Accordingly, to identify treatments with high efficacy, the following review focuses on those interventions that have been reported to be successful in multiple randomized, double-blind studies.

Potassium-containing dentifrices have been found to be effective in a majority of randomized, double-blind clinical trials. The placebo controls in these studies consisted of the dentifrice without the potassium nitrate ($KNO_3$) component. In a review of the literature, 73% of formulations of potassium nitrate–containing dentifrices were significantly better than the placebo at reducing dentinal hypersensitivity incited by the application of cold air pulses (Orchardson and Gillam) (Table 9-1). When analyzed on a sample-weighted basis, the overall results indicated that placebo dentifrices reduced sensitivity to cold by about 35%; 3.75% $KNO_3$ dentifrices reduced sensitivity to cold by about 60.8%; and 5% $KNO_3$ dentifrices reduced sensitivity to cold by about 61.2% (see Table 9-1). This finding is clinically relevant because cold is reported to be the most common stimulus of dentinal hypersensitivity (Rees, Gillam and Newman). Moreover, 64% of formulations of

**TABLE 9-1** Efficacy of various potassium nitrate dentifrice formulations on cold air–induced dentinal hypersensitivity

| Study (year) | % active agent ($KNO_3$) | N | Duration (wk) | Active (%)‡ | Placebo (%)‡ | Significance (*P* value) |
|---|---|---|---|---|---|---|
| Tarbet et al (1980) | 5 | 27 | 4 | 65 | 20 | < .05 |
| Manochehr-Pour et al (1984)† | 5 | 75 | 12 | 63 | 37 | NS |
| Manochehr-Pour et al (1984)† | 5 | 75 | 12 | 52 | 37 | NS |
| Silverman (1985)† | 5 | 68 | 12 | 75 | 40 | < .001 |
| Silverman (1985)† | 5 | 68 | 12 | 78 | 40 | < .001 |
| Salvato et al (1992) | 3.75 | 41 | 12 | 66 | 32 | .001 |
| Nagata et al (1994) | 5 | 36 | 12 | 80 | 27 | < .01 |
| Schiff et al (1994) | 5 | 60 | 12 | 61 | 0 | < .001 |
| Silverman et al (1994)† | 3.75 | 62 | 8 | 66 | 28 | < .05 |
| Silverman et al (1994)† | 3.75 | 62 | 8 | 61 | 28 | < .05 |
| Silverman et al (1996) | 5 | 220 | 8 | 54 | 30 | < .001 |
| Gillam et al (1996) | 3.75 | 56 | 6 | 51 | 48 | NS |
| West et al (1997) | 5 | 112 | 6 | 48 | 53 | NS |
| Schiff et al (1998) | 5 | 39 | 8 | 82 | 40 | < .0001 |

Data from Orchardson and Gillam.

†Studies in which different formulations were compared have separate listings.

‡Percentage reduction in sensitivity to cold air stimulus from baseline values.

NS = not significant.



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potassium nitrate–containing dentifrices were significantly better than the placebo for overall global reduction in dentinal hypersensitivity symptoms (Table 9-2). Thus, the majority of randomized controlled clinical trials indicate that potassium-containing dentifrices are effective in reducing dentinal hypersensitivity (Poulsen et al, Hu et al 2004, Salvato et al, Orchardson and Gillam). A recent study conducted by the National Dental Practice-Based Research Network reports that the most common single recommendation for dentinal hypersensitivity by the network practitioners is potassium nitrate toothpaste (Kopycka-Kedzierawski et al).

The efficacy of other agents for reducing dentinal hypersensitivity has also been evaluated in clinical trials. For example, 10% strontium chloride ($SrCl_2$) has been evaluated in numerous studies. In two-cell studies (ie, $SrCl_2$ versus placebo dentifrice), the weighted mean efficacy of $SrCl_2$ was a 72.5% reduction in hypersensitivity, while the mean efficacy of the placebo dentifrice was a 34.3% reduction in hypersensitivity (Orchardson and Gillam). However, in studies consisting of three or more cells (ie, at least two active groups and a placebo dentifrice), the weighted mean efficacy of $SrCl_2$ was only a 51.8% reduction in hypersensitivity, while the mean efficacy of the placebo dentifrice was a 41.0% reduction in hypersensitivity. When the results of both types of study are combined, $SrCl_2$ produced about a 50% reduction in dentinal hypersensitivity. Clearly, experimental design issues contribute to the variations in efficacy estimates for desensitizing dentifrices. Furthermore, the efficacy of fluoride-containing medicaments on dentinal hypersensitivity has been evaluated in several clinical trials. Fluoride-containing dentifrices also have been reported to reduce dentinal hypersensitivity in some but not all studies (Plagmann et al, Bolden et al).

**TABLE 9-2** Efficacy of various potassium nitrate dentifrice formulations on patients' global subjective ratings of dentinal hypersensitivity

| Study (year) | % active agent ($KNO_3$) | N | Duration (wk) | Active (%)‡ | Placebo (%)‡ | Significance (*P* value) |
|---|---|---|---|---|---|---|
| Tarbet et al (1980) | 5 | 27 | 4 | 92 | 21 | < .001 |
| Manochehr-Pour et al (1984)† | 5 | 75 | 12 | 54 | 60 | NS |
| Manochehr-Pour et al (1984)† | 5 | 75 | 12 | 36 | 60 | NS |
| Silverman (1985)† | 5 | 68 | 12 | 71 | 36 | < .001 |
| Silverman (1985)† | 5 | 68 | 12 | 75 | 36 | < .001 |
| Salvato et al (1992) | 3.75 | 41 | 12 | 75 | 23 | < .001 |
| Nagata et al (1994) | 5 | 36 | 12 | 82 | 28 | < .01 |
| Schiff et al (1994) | 5 | 60 | 12 | 52 | 30 | < .01 |
| Silverman et al (1994)† | 3.75 | 62 | 8 | 61 | 32 | < .10 |
| Silverman et al (1994)† | 3.75 | 62 | 8 | 52 | 32 | < .05 |
| Silverman et al (1996) | 5 | 220 | 8 | 55 | 29 | < .001 |
| Gillam et al (1996) | 3.75 | 56 | 6 | 54 | 29 | < .001 |
| West et al (1997) | 5 | 112 | 6 | 54 | 43 | NS |
| Schiff et al (1998) | 5 | 39 | 8 | 30 | 19 | NS |

Data from Orchardson and Gillam.

†Studies in which different formulations were compared have separate listings.

‡Percentage reduction in patients' subjective ratings of overall dentinal sensitivity compared with baseline values.

NS = not significant.

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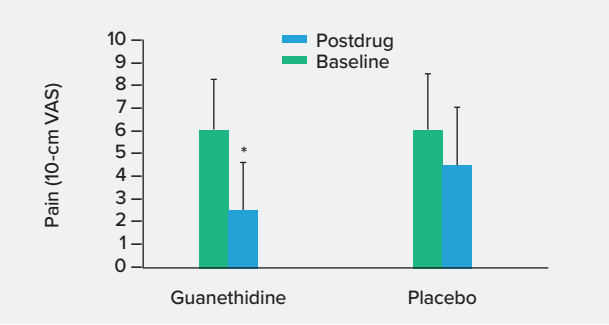


▲ **FIG 9-7** Effect of topical application of a 1% solution of guanethidine or placebo on dentinal hypersensitivity to cold air stimulation. VAS = visual analog scale. N = 39 patients; *$P$ < .05 versus placebo posttreatment. (Data from Dunne and Hannington-Kiff.)

Guanethidine has also been evaluated for the management of dentinal hypersensitivity. In a randomized, double-blind study, the application of a 1% solution of guanethidine to exposed dentinal tubules produced about a 50% reduction in sensitivity compared with baseline values (Dunne and Hannington-Kiff) (Fig 9-7). Guanethidine acts by inhibiting exocytosis from peripheral terminals of sympathetic fibers and has been shown previously to block sympathetically regulated blood flow in the dental pulp (Raab et al). However, guanethidine may also alter the activity of nociceptors. The application of guanethidine to human skin increases pricking pain thresholds, an effect not observed with other vasodilators. Mashimo et al concluded that guanethidine directly alters human nociceptors, a process that may involve Aδ-nociceptors, because these neurons are thought to encode for pricking pain sensation. Other studies have suggested that guanethidine also acts on the capsaicin-sensitive class of unmyelinated nociceptors (Zheng et al). Collectively, these studies suggest that further research is warranted on the potential application of guanethidine for treating dentinal hypersensitivity.

Advances in the management of dentinal hypersensitivity include the use of low-level lasers (Tolentino et al, Barros et al). The results of two recent meta-analyses suggest that the use of lasers on teeth with dentinal hypersensitivity results in a reduction in pain evoked by tactile stimuli and air blasts (Mahdian et al, Shan et al). However, that evidence is limited, and further clinical investigations are warranted.

In summary, dentinal hypersensitivity represents a common, widespread form of odontogenic pain that is believed to be mediated primarily by the activation of neurons that innervate dentinal tubules. The diagnosis of dentinal hypersensitivity is based on the eliciting stimuli, the duration and location of the pain, and the absence of pulpal (ie, symptoms) or radiographic (ie, changes seen) pathoses. The management of dentinal hypersensitivity involves the application of therapies that reduce either the flow of dentinal fluid (see also chapter 3) or the activity of dentinal neurons.

In addition, patients should be advised to avoid habits or agents that increase dentinal permeability. For example, studies have reported that tartar-control dentifrices and toothbrushing in the presence of dietary acids or certain mouthwashes (eg, those containing hexetidine or fluoride/antiseptic) may increase the risk of dentinal sensitivity by removing the smear layer of exposed dentinal tubules (Lavigne et al, Addy 2005, Absi et al).

## Management of Pulpitis and Related Pain Conditions

Another common form of odontalgia is the result of pulpitis or periradicular pain pathoses. For the purposes of this chapter, this form of odontalgia includes pain resulting from activation of pulpal or periradicular nociceptors. It is appropriate to include periradicular nociceptors in this category because the accumulation of bacteria and bacterial byproducts from necrotic root canal systems constitutes the predominant etiologic factor for periradicular inflammation and pain, which is another good example of the relationship between dental pulp and other tissues during disease. Thus, relevant clinical diagnoses will include odontogenic pain of inflammatory etiologies, such as irreversible pulpitis and pulpal necrosis with acute apical periodontitis.

This section reviews both pharmacologic and nonpharmacologic methods for pain control. The clinician must use an integrated approach in combining these methods to control dental pain, which has been called the *3D method* for pain control: diagnosis, definitive dental treatment (nonpharmacologic methods), and drugs (pharmacologic methods). This approach is summarized in Box 9-1.

**BOX 9-1 3D method for managing acute odontogenic pain**

- Diagnosis
- Definitive dental treatment
  - Pulpotomy, pulpectomy
  - Extraction
  - Incision for drainage
- Drugs
  - Pretreat with nonsteroidal anti-inflammatory drugs (NSAIDs) or acetaminophen when appropriate
  - Prescribe medications to be taken "by the clock" rather than "as necessary"
  - Use long-acting local anesthetics when indicated
  - Use a flexible prescription plan

Based on Keiser and Byrne.


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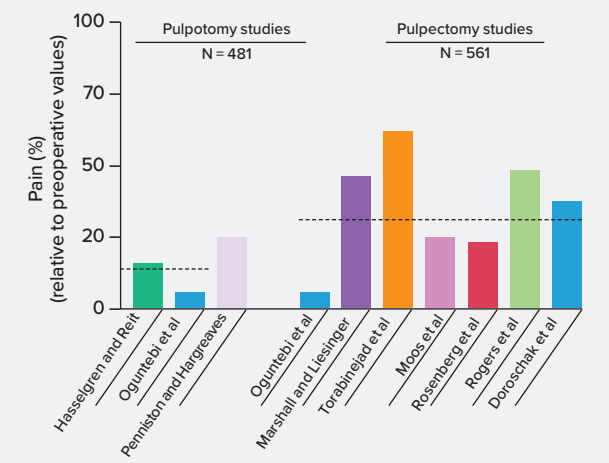
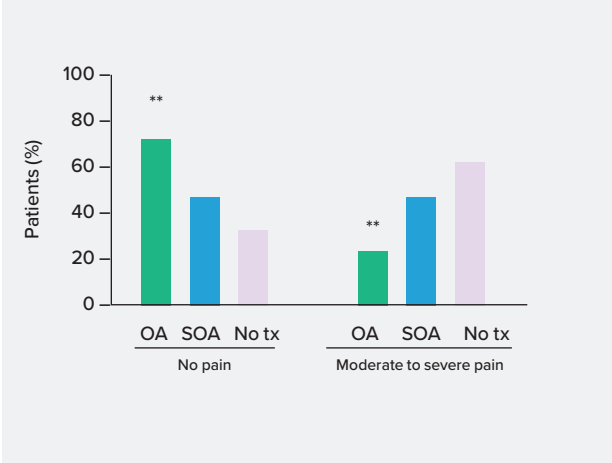



▲ **FIG 9-8** Effects of pulpotomy and pulpectomy on endodontic-related pain. Preoperative pain values are normalized to 100%. The two *dashed horizontal lines* represent the sample size–weighted mean reduction in pain for the pulpotomy and pulpectomy groups. (Data from Hargreaves and Baumgartner.)




▲ **FIG 9-9** Effect of occlusal adjustment (OA), simulated occlusal adjustment (SOA, by reduction of nonfunctional cusp), or no treatment (No tx) on the incidence of posttreatment pain in 117 endodontic patients. $^{**}P < .01$. (Redrawn after Rosenberg et al.)

## Diagnosis of odontalgia

Diagnosis is of obvious importance in managing acute pain because effective treatment is directed at removing the etiology of pain. For example, nitroglycerin is effective for reducing anginal pain, yet it has comparatively little analgesic activity in most other pain conditions. Similarly, if a patient has pain associated with an abscess, then an incision for drainage may prove effective in reducing pain. In both examples, the treatments are effective because they reduce the etiologic factors that elicit the pain.

For patients with acute pain, an accurate diagnosis generally leads to predictable treatment strategies, but for patients with chronic pain, the etiologies of pain are less well understood and probably multifactorial. The first step in treating the acute pain patient is establishing the diagnosis. The clinician should be aware of differential diagnoses for patients who present with a chief complaint of tooth pain because etiologies, treatment strategies, and prognoses vary considerably (see chapter 15).

## Nonpharmacologic methods of managing odontogenic pain

Numerous studies indicate that definitive dental treatment alone provides predictable and substantial relief from odontogenic pain. In patients with irreversible pulpitis, pulpotomy treatment, regardless of the coronal medicament used, has been reported to eliminate pain symptoms in 88% of patients evaluated 1 day after treatment. The efficacy of definitive dental treatment for reducing acute pain symptoms has been confirmed by many other studies. Figure 9-8 summarizes the results from more than 1,000 patients in clinical trials to evaluate the efficacy of pulpotomy or pulpectomy for relieving odontogenic pain (Hargreaves and Baumgartner). Because pulpotomies are only performed for vital endodontic cases (eg, pain of pulpal origin), whereas pulpectomies are performed to manage more complex pain conditions (ie, pain of pulpal and periradicular origin), it is not surprising that pulpotomies appear more effective than pulpectomies for relieving pain (Oguntebi et al, Hasselgren and Reit, Moos et al).

Other forms of definitive dental treatment include occlusal adjustment and incision for drainage procedures. In one study of 117 patients, about twice as many patients who underwent occlusal adjustment reported no posttreatment pain compared to no-treatment control subjects (Rosenberg et al) (Fig 9-9). The authors concluded that occlusal adjustment was particularly effective for reducing pain in patients who had vital pulps and percussion sensitivity. These findings were replicated in a more recent study on patients with irreversible pulpitis and mild tenderness to percussion (Parirokh et al 2013).

Another nonpharmacologic approach is to reduce intraosseous pressure. Although trephination has been advocated to reduce pain after endodontic therapy, several randomized clinical trials have failed to demonstrate a significant difference between the effect of trephination with pulpectomy and the effect of pulpectomy alone (Houck et al, Nist et al). Thus, this technique does not appear to result in a clinically reproducible reduction in inflammatory mediators or interstitial pressure.

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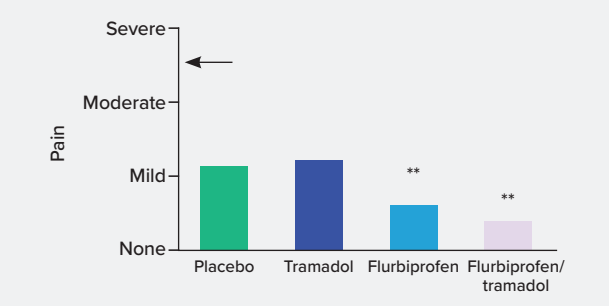
The pain-relieving benefits of these definitive dental treatments are believed to be based on a reduction in tissue levels of factors that stimulate peripheral terminals of nociceptors or a reduction in the mechanical stimulation of sensitized nociceptors (eg, occlusal adjustment). Effective chemomechanical debridement of the infected root canal system combined (when indicated) with incision for drainage procedures provides predictable pain reduction in patients with an endodontic emergency. Of course, if the tooth has a hopeless prognosis, extraction will also reduce pain by reducing tissue levels of these factors. From this perspective, it can be concluded that treating the unscheduled emergency patient with drugs alone is not a definitive intervention. Instead, pharmacologic management of pain should be considered with definitive dental treatment as a combined therapeutic approach to managing odontogenic pain.

Although space limitations preclude a more thorough review, other nonpharmacologic approaches have been evaluated for reduction of dental pain. These approaches include cognitive, motivational, and affective interventions and range from simple approaches such as establishing a confident doctor-patient relationship to more complex procedures. Several nonpharmacologic approaches such as hypnosis may be considered as a component of the overall strategy of managing dental inflammatory pain.




▲ **FIG 9-10** Comparison of flurbiprofen (100-mg loading dose, then 50 mg every 6 h), tramadol (100-mg initial dose, then 100 mg every 6 h), and the combination of flurbiprofen and tramadol (same dosages) to placebo in endodontic patients. Patients received local anesthesia, pulpectomies, and drugs. Pain levels were reported 24 hours after treatment. *Arrow* shows mean preoperative pain level. N = 11 to 12 per group; $^{**}P < .01$ versus placebo. (Redrawn after Doroschak et al.)

## Pharmacologic methods of managing odontogenic pain

Three primary pharmacologic approaches to odontogenic pain control are reviewed in this section: *(1)* drugs that block inflammatory mediators that sensitize or activate pulpal nociceptors, *(2)* drugs that block the propagation of impulses along peripheral nerves, and *(3)* drugs that block central mechanisms of pain perception and hyperalgesia.

### Drugs that block inflammatory mediators that sensitize or activate pulpal nociceptors

Although numerous clinical pharmacology studies have evaluated analgesics for the treatment of dental pain, the majority of these studies employ a clinical model of acute inflammatory pain elicited by surgical trauma. Acute surgery-induced inflammation is mediated largely by the release of eicosanoids such as prostacyclin and prostaglandin $E_2$ ($PGE_2$), as well as other inflammatory mediators such as bradykinin. In contrast, pulpal and periradicular pain is often associated with chronic inflammation, characterized by the presence of bacterial byproducts, an influx of immune cells with activation of the cytokine network, and other inflammatory mediators. These considerations suggest that the composition and concentrations of inflammatory mediators that activate and sensitize nociceptors likely differ in these two models of orofacial pain. Moreover, the chronicity of pulpal inflammation permits sprouting of nociceptor terminals, and thus the peripheral anatomy of the pain system changes during pulpal inflammation. Therefore, it is likely that the relative efficacy of analgesics for the treatment of surgery-induced pain and pulp-related pain differs. Accordingly, this review focuses on clinical trials of endodontic pain, incorporating clinical trials of surgery-induced pain only to illustrate additional concepts.

One major class of drugs for managing endodontic pain are the non-narcotic analgesics, which include both nonsteroidal anti-inflammatory drugs (NSAIDs) and acetaminophen. These drugs produce analgesia by actions in both the peripherally inflamed tissue as well as in certain regions in the brain and spinal cord. NSAIDs have been shown to be very effective for managing pain of inflammatory origin that arises from either surgery-induced trauma or pulpal and periradicular pain. Indeed, the results of a systematic review and meta-analysis show that NSAIDs effectively reduce postoperative pain in endodontic patients (Smith et al). Several randomized, double-blind, placebo-controlled clinical trials in endodontic pain patients indicate that ibuprofen (400 mg), ketoprofen (50 mg), flurbiprofen (100 mg), and ketorolac (30 to 60 mg) all produce significant analgesia compared to a placebo medication (Penniston and Hargreaves, Curtis et al, Doroschak et al) (Figs 9-10 and 9-11).

In interpreting these studies, it is important to realize that endodontic treatment alone (eg, pulpectomy) has a major effect on reducing pain regardless of pharmacologic treatment (see Fig 9-8). This reduction in posttreatment pain combined with variable levels of preoperative pain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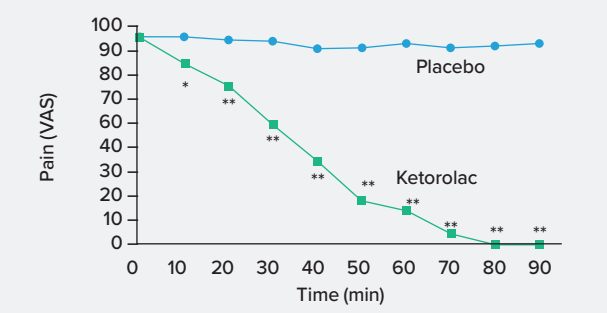
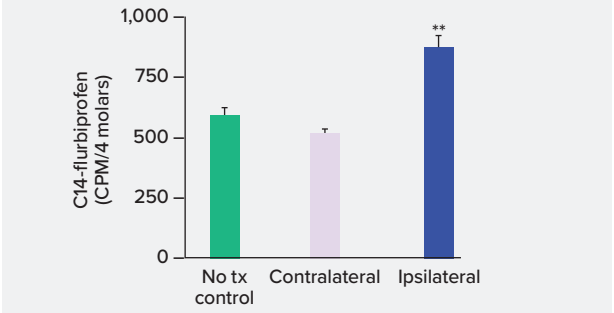



▲ **FIG 9-11** Comparison of ketorolac (60-mg intramuscular injection) to placebo for relief of endodontic pain. Patients (N = 40) completed a baseline visual analog scale (VAS) for pain, received a drug injection, and then completed postdrug pain scales. No endodontic treatment or local anesthetic was provided. *$P$ < .05 versus placebo; **$P$ < .01 versus placebo. (Redrawn after Curtis et al.)




▲ **FIG 9-12** Preferential distribution of NSAIDs into inflamed pulp. Rats were anesthetized and underwent either no treatment (No tx) or pulpal exposure of the mandibular first molar. Seven days later, rats were anesthetized and received an intravenous injection of C14-flurbiprofen. No-treatment control (from a separate group of animals), contralateral (no pulpal exposure), and ipsilateral (pulpal exposure) teeth were extracted, homogenized, and analyzed for C14-flurbiprofen. CPM = counts per minute. N = 7 rats per group; **$P$ < .01 versus both the contralateral and no-treatment control groups. (Redrawn after Bunczak-Reeh and Hargreaves.)

reduces the statistical power of endodontic clinical trials for detecting active analgesics over time or in all patient groups (the so-called "floor effect"). This limitation causes a problem in interpreting clinical pain studies in general and may explain why some endodontic clinical trials fail to detect analgesic treatment or only detect it in those patients with moderate to severe preoperative pain.

Although many NSAIDs are available in the marketplace, comparatively few endodontic studies have directly compared one NSAID with another to determine analgesia and side-effect liability. Ibuprofen salts including ibuprofen lysine, ibuprofen arginine, and ibuprofen sodium dihydrate are more soluble than ibuprofen acid, allowing greater absorption, higher drug plasma concentration, and lower time to reach the maximum concentration of the drug than ibuprofen acid. This results in faster and more profound analgesia (Moore et al). In a randomized, double-masked study on patients diagnosed with symptomatic irreversible pulpitis and symptomatic apical periodontitis and experiencing moderate to severe pain, the administration of ibuprofen sodium dihydrate resulted in a faster onset of 50% pain relief as compared to ibuprofen acid (26.5 min versus 44 min) (Taggar et al). Ibuprofen sodium dihydrate also provided a greater reduction in spontaneous pain and in mechanical allodynia.

Ibuprofen is generally considered the prototype NSAID and has a well-documented efficacy and safety profile (Dionne et al). NSAIDs have a relatively high affinity to plasma proteins, and they are preferentially distributed to inflamed tissue by local vasodilation and plasma extravasation (Bunczak-Reeh and Hargreaves). Thus, NSAIDs are preferentially distributed into inflamed dental pulp compared with control dental pulp (Fig 9-12). This quality may improve the relative efficacy of NSAIDs compared with other analgesic drug classes. It is also important to note that the cyclooxygenase enzyme COX-2 is elevated in inflamed human dental pulps as compared with control pulpal tissue, and this may lead to a greater analgesic efficacy of COX-2 inhibitors in pulpitis (Nakanishi et al). Celecoxib, the prototype selective COX-2 inhibitor, is the only selective COX-2 inhibitor that is approved by the US Food and Drug Administration (FDA) for acute pain.

A serious side effect observed with the use of selective COX-2 inhibitors is the increase of prothrombotic events, resulting in increased risk for mortality and morbidity. A prospective, population-based cohort study of patients (N= 7,636) taking either nonselective NSAIDs or COX-2–selective NSAIDs found that the mean hazard ratio for experiencing ischemic stroke was 4.52 for patients taking the selective COX-2 inhibitor rofecoxib (Vioxx, Merck) compared to 1.68 for nonselective NSAIDs (Haag et al). The demonstration of an increased risk for prothrombotic events following long-term administration of rofecoxib in multiple studies led to the withdrawal of this drug from the US market in 2004 (Psaty and Kronmal). The mechanism of NSAID-associated increase in stroke risk is thought to be related to the inhibition of prostacyclin, resulting in vasoconstriction, increased hypertension due to renal dysregulation, and increased platelet aggregation due to increased production of thromboxane $A_2$ (Park and Bavry). This increased risk for prothrombotic events in susceptible individuals has also been observed with nonselective NSAIDs including ibuprofen, naproxen, and diclofenac (Bally et al).

Another adverse effect of NSAIDs is the risk of serious or fatal gastrointestinal bleeding (Woodcock). The risk of

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developing an NSAID-associated gastrointestinal complication is highest among patients taking concurrent aspirin, steroid, or warfarin; patients with a previous history of an ulcer hemorrhage; and patients older than 65 years (Spiegel et al, Laine et al). One of the strategies used to prevent gastrointestinal bleeding is to use a proton pump inhibitor with an NSAID. A combination of a proton pump inhibitor, esomeprazole magnesium, and naproxen (Vimovo, AstraZeneca) is now available. While this is a cost-effective approach, it does not protect the lower gastrointestinal tract (Spiegel et al). Another approach is to use a histamine (H2) receptor antagonist in combination with an NSAID. The combination of famotidine (an H2 receptor antagonist) and ibuprofen (Duexis, Horizon Pharma) was recently cleared by the FDA. A third approach is to use a selective COX-2 inhibitor instead of an NSAID.

Although space limitations preclude an extensive review of contraindications for NSAIDs, the clinician should be aware of these issues and understand that acetaminophen, either alone or in an acetaminophen-opioid combination, may represent an alternative for patients unable to take NSAIDs. Sites for evaluating newly released analgesics as well as potential adverse effects, contraindications, and drug interactions include www.rxlist.com, www.pharminfo.net, and www.epocrates.com, among others.

Acetaminophen (N-acetyl-p-aminophenol), used alone or in combination with an NSAID or a narcotic, is also used for pain relief. In a study evaluating pain following pulpectomy, the combination of acetaminophen (1,000 mg) and ibuprofen (600 mg) was shown to provide greater pain relief than ibuprofen (600 mg) alone (Menhinick et al). Approximately 184 acetaminophen compounds are available as over-the-counter or prescription drugs.

The widespread use of acetaminophen has resulted in a substantial increase in the number of cases of acute liver toxicity. The factors known to contribute to acetaminophen liver toxicity are listed in Box 9-2. The FDA has taken new steps to reduce the risk of liver injury associated with the use of acetaminophen. These include limiting the maximum amount of acetaminophen in prescription acetaminophen combination products to 325 mg per tablet, capsule, or other dosage unit and requiring a boxed warning on all prescription acetaminophen products to highlight the potential risk for severe liver injury. Because the use of medications to manage endodontic pain is limited to a few days, the risk for acetaminophen-related liver damage is relatively low in endodontics.

An effective analgesic strategy that enhances the analgesic effect of acetaminophen while limiting its dose (and adverse effects) is to combine it with an NSAID. Evidence from multiple studies shows that this combination produces nearly twice the analgesic response compared to an NSAID alone in patients experiencing acute postsurgical pain (Breivik et al, Cooper, Menhinick et al). This effect is also seen in endodontic patients in whom the administration of ibuprofen (600 mg) with acetaminophen (1,000 mg) produced significant relief of postendodontic pain as compared to ibuprofen alone. The concurrent administration of acetaminophen and NSAIDs has a synergistic effect and is well tolerated, with no detectable increase in side effects (Breivik et al, Lanza, Stambaugh and Drew, Wright). The FDA has recently approved an over-the-counter ibuprofen and acetaminophen combination drug marketed as Advil Dual Action (GlaxoSmithKline). This drug combination contains 250 mg of ibuprofen plus 500 mg of acetaminophen. This drug combination poses some challenges in a flexible prescription strategy. If greater analgesia is needed, the use of more than one dose every 6 hours would exceed the FDA recommendation for the daily dose of acetaminophen. Thus, the use of both drugs separately (to be taken concomitantly) allows for greater titration and flexibility to provide a customized analgesic regimen.

Steroids, or more properly glucocorticoids, form an additional class of drugs that interfere with the production or release of mediators that activate or sensitize nociceptors. Glucocorticoids are known to reduce the inflammatory response by suppressing vasodilation, neutrophil migration, and phagocytosis and by inhibiting the formation of arachidonic acid from neutrophil and macrophage cell membrane phospholipids, thereby blocking the cyclooxygenase and lipoxygenase pathways and respective synthesis of prostaglandins and leukotrienes. An animal study on the anti-inflammatory effects of corticosteroids on inflamed periradicular tissues demonstrated that dexamethasone significantly reduced the number of neutrophils present and thus had an anti-inflammatory effect on the periradicular tissues of the teeth in which endodontic treatment had been performed (Nobuhara et al).

**BOX 9-2 Factors associated with increased risk of acetaminophen-induced liver toxicity**

- Ingestion of more than 4 g of acetaminophen within a 24-hour period (this includes unintentional overdose in patients taking multiple acetaminophen combinations for different conditions and patients taking brand names of acetaminophen-containing products that do not definitively disclose that acetaminophen is an ingredient)
- Fasting or malnourishment
- Preexisting liver disease
- Genetic predisposition to liver injury
- Alcohol abuse
- Concomitant use of phenytoin, isoniazid, some protease inhibitors such as ritonavir, or zidovudine

Information from Schilling et al and Guggenheimer and Moore.


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Several clinical trials have evaluated the efficacy of glucocorticoids for reducing postendodontic pain after intracanal, oral, or intramuscular administration. In general, intracanal steroids appear to provide the most consistent reductions in postoperative pain or flare-ups when used in vital teeth. The reduced efficacy reported in necrotic teeth may be due to *(1)* poor absorption via this route of administration, *(2)* diffusion of inadequate amounts of the drug into the periradicular tissue, or *(3)* other factors such as experimental design or statistical power.

Studies have evaluated the effects of systemic administration of corticosteroids on postoperative pain or flare-ups. For example, a double-blind, parallel-design clinical trial reported that pretreatment with a single oral dose of prednisolone reduced postendodontic pain for up to 24 hours in teeth with vital and necrotic pulps (Jalalzadeh et al). Administration of oral dexamethasone significantly reduced postoperative pain when compared to a placebo at 8 and 24 hours (Krasner and Jackson). A subsequent study on the effect of a larger oral dose of dexamethasone (ie, 12 mg dexamethasone given every 4 hours) on the severity of posttreatment endodontic pain provides further evidence on the analgesic effectiveness of the drug at 8 hours (Glassman). However, there was no effect on the severity of pain at 24 and 48 hours after treatment. In another randomized clinical trial evaluating submucosal injection of dexamethasone in pulpitis patients undergoing endodontic therapy, patients in the steroid group reported less pain up to 48 hours posttreatment (Aksoy and Ege). In general, these studies indicate that systemically administered corticosteroids reduce the severity of posttreatment endodontic pain compared to placebo treatment (Marshall and Walton) (Fig 9-13).

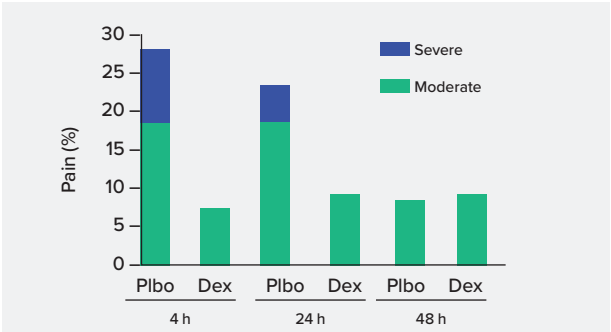
As described earlier, the lack of a drug effect at later time points may be due in part to the pain-relieving effects of endodontic instrumentation. In support of this point, a study on the effects of intraosseous steroid injection on pain in patients with untreated pulpitis demonstrated that steroids produced a significant reduction in pulpitis pain over a 7-day observation period (Gallatin et al) (Fig 9-14). Thus, steroids can elicit long-term reduction in pulpal pain in patients who receive no endodontic treatment. Interestingly, 95% of steroid-treated dental pulps and 81% of the placebo-treated dental pulps were still vital at the 7-day observation.

Taken together, these studies indicate that drugs that suppress the release or actions of inflammatory mediators on nociceptors have an analgesic effect on pain caused by inflammation of pulpal and periradicular tissue. The design of a pain-management plan is considered later in this chapter.

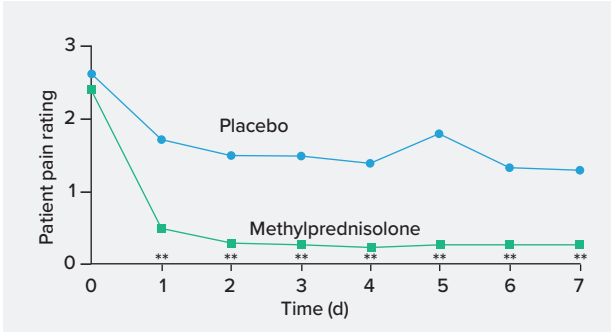
### Drugs that block propagation of impulses along peripheral nerves

Local anesthetics represent an important component of pain control. Local anesthetics offer the benefit of prolonged pain control following completion of the endodontic appointment; for example, long-acting local anesthetics etidocaine and bupivacaine offer prolonged pain control 6 hours or more after injection (Dunsky and Moore, Moore and Hersh). Furthermore, research on pain mechanisms has revealed the existence of a central component to hyperalgesia, which can be established by an intense barrage of activity from peripheral nociceptors, particularly the unmyelinated C-nociceptors. The preemptive analgesia hypothesis states that clinical pain is reduced when the peripheral barrage of nociceptors is reduced (Woolf and Costigan).

In a clinical study, patients given an inferior alveolar nerve block (IANB) injection with bupivacaine prior to an oral surgical procedure reported less pain 24 and 48 hours after the procedure than did placebo-injected




▲ **FIG 9-13** Comparison of dexamethasone (Dex) (4-mg intramuscular injection) to placebo (Plbo) for pain after endodontic instrumentation. N = 50; ***P* < .01 versus placebo. (Redrawn after Marshall and Walton.)




▲ **FIG 9-14** Comparison of the effects of methylprednisolone (40-mg intraosseous injection) to placebo for relief of pain due to irreversible pulpitis. After diagnosis and drug injection, patients reported pain levels for 7 days before any endodontic treatment was started. N = 40; ***P* < .01 versus placebo. (Redrawn after Gallatin et al.)

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patients (Gordon et al). A randomized clinical trial on patients undergoing single-visit root canal treatments also reported that patients who received IANB injection with bupivacaine had less posttreatment pain than those who received an IANB injection with lidocaine (Parirokh et al 2012). This result is not limited to extraction procedures; infiltration with bupivacaine prior to tonsillectomy reduced postoperative pain for 7 days compared with patients given placebo injections (Jebeles et al). Collectively, these studies indicate that measures that reduce the peripheral barrage of nociceptors, such as long-acting local anesthetics, should be considered for treatment of endodontic pain and that they may reduce posttreatment pain even days after a single administration.

Factors that affect the success of local anesthesia include the type of local anesthetic used, the speed of injection, the route of administration, and the presence of inflammation. Data from several clinical trials demonstrate that lidocaine provides predictable success when used for maxillary infiltration, IANB, or intraosseous injections (Dunbar et al, Nusstein et al 2002, Dagher et al). A randomized, double-blind clinical trial on patients with symptomatic irreversible pulpitis reported a higher success rate of pulpal anesthesia using a combination of lidocaine with clonidine as compared to lidocaine with epinephrine (59% versus 29%) (Shadmehr et al). This novel combination may result in less postoperative pain and thus may be a better alternative to lidocaine with epinephrine for surgical procedures (Brkovic et al).

Another commonly used anesthetic that is comparable to lidocaine is 3% mepivacaine. A randomized, double-blind clinical study compared IANBs with 3% mepivacaine, 4% prilocaine, and 2% lidocaine with 1:100,000 epinephrine (McLean et al). No statistically significant differences were detected in the onset, success or failure, or duration of pulpal anesthesia among the three solutions.

Articaine has a reputation for providing improved local anesthetic effect. However, it is yet to be clearly demonstrated that the use of articaine results in greater magnitude or duration of anesthesia than lidocaine. A randomized, double-blind crossover study in volunteers with normal pulps demonstrated that the effects of 4% articaine with 1:100,000 epinephrine did not differ from 2% lidocaine with 1:100,000 epinephrine when used for IANBs (Mikesell et al). In a study comparing the analgesic efficacy of the two solutions when administered as a supplemental buccal infiltration following IANB with 4% articaine with 1:100,000 epinephrine, the success of pulpal anesthesia—defined as no response to the maximum output of an electrical pulp tester—was greater with 4% articaine with 1:100,000 epinephrine (88%) than with 2% lidocaine with 1:100,000 epinephrine (71%) (Haase et al). Furthermore, a meta-analysis comparing articaine and lidocaine given by different routes (infiltration or blocks) concluded that articaine had a higher success rate than lidocaine only when administered as a supplemental injection given after IANB in symptomatic teeth (Smith et al).

The speed of injection may also influence the success of pulpal anesthesia. In a randomized, double-blind, crossover clinical trial evaluating the efficacy of IANBs, slow injection (60 seconds) of 2 mL of 2% lidocaine with 1:80,000 epinephrine resulted in a higher success rate of pulpal anesthesia than fast injection (15 seconds). Although this study was conducted on teeth with normal pulps, the success rate was not 100% (Kanaa et al). A prior similar study by the same group evaluating the speed of injection for incisive/mental nerve block showed no difference in the effectiveness based on speed (Whitworth et al). The reason for this discrepancy is not clear but may be related to differences in experimental design (eg, differences in injection volume).

The rate of failure of local anesthetics is higher in inflamed tissues than in normal tissues. Inflammatory mediators activate or sensitize peripheral nociceptors and thus contribute to the peripheral mechanisms of reduced pain threshold (allodynia) or increased responsiveness to painful stimuli (hyperalgesia). Mediators such as $PGE_2$, nerve growth factor, and serotonin increase the activity of tetrodotoxin-resistant sodium channels (Gold et al, Rush and Waxman). Because these channels are only one-quarter as sensitive to lidocaine as other sodium channels, their increased activity during inflammation is thought to account, in part, for the failure of local anesthetics in inflamed tissues (Rush and Waxman). Because $PGE_2$ is thought to play a key role in sensitizing this channel, it is possible that NSAIDs enhance the efficacy of local anesthetics by reducing $PGE_2$-mediated channel phosphorylation.

Several important pharmacokinetic and pharmacodynamic characteristics of intraosseous local anesthetic injection have been determined using volunteers with normal pulps in whom pulpal anesthesia was measured with an electric pulp tester. Intraosseous injection of 1.8 mL of 2% lidocaine with 1:100,000 epinephrine provided significant anesthesia for 74% of first molars (Replogle et al 1997). The dosage of 2% lidocaine with epinephrine produced a transient (4-minute) period of tachycardia with an average increase in heart rate of 28 beats per minute (Replogle et al 1999). The intraosseous administration of a 1.8-mL volume of 3% mepivacaine produced a 45% anesthesia rate in mandibular first molars, and anesthetic success increased with a second injection of 3% mepivacaine (Replogle et al 1997, Reisman et al). The intraosseous injection of 3% mepivacaine had no significant cardiovascular effects (Replogle et al 1999). About 5% of patients had delayed healing at the site of injection (Reisman et al).


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Additional studies have evaluated the efficacy of a supplemental intraosseous injection of local anesthetics in clinical patients with pain caused by irreversible pulpitis (Nusstein et al 1998, Reisman et al). In one study of 48 patients with irreversible pulpitis in mandibular teeth, an IANB of 2% lidocaine with 1:100,000 epinephrine was only 25% successful for pulpal anesthesia, as defined by a negative response to the electrical pulp tester and patients' ratings of no or only mild pain upon endodontic access; a supplementary intraosseous injection of 3% mepivacaine increased anesthetic success to 80% (Reisman et al). In a parallel study of 51 patients with irreversible pulpitis of mandibular teeth, an IANB of 2% lidocaine with 1:100,000 epinephrine was only 19% successful for pulpal anesthesia; a supplementary injection of 2% lidocaine with 1:100,000 epinephrine increased anesthetic success to 91% (Nusstein et al 1998). Similar results have been observed in another clinical trial of irreversible pulpitis in maxillary and mandibular teeth (Reisman et al). The supplemental intraosseous injection of 1.8 mL of 4% articaine with 1:100,000 epinephrine was 86% successful for pulpal anesthesia in teeth with irreversible pulpitis. Collectively, these studies indicate that intraosseous injection of 3% mepivacaine, 2% lidocaine with 1:100,000 epinephrine, or 4% articaine with 1:100,000 epinephrine can lead to a threefold to fourfold improvement in anesthetic success in patients with irreversible pulpitis.

Space limitations preclude a detailed review of the technique for intraosseous injection or other routes of injection and the relative indications and contraindications of various local anesthetics. The clinician is encouraged to seek this information in one of the many excellent clinical texts available.

Intraligamentary injections are another route for administration of local anesthetics. A randomized, double-blind crossover study compared intraligamentary injections of 2% lidocaine with 1:100,000 epinephrine and 4% articaine with 1:100,000 epinephrine in volunteers with normal pulps (Nusstein et al 2004). The incidence of moderate pain was 14% to 27% during needle insertion and 8% to 18% during solution deposition. On the day after the injection, 20% to 31% of subjects reported moderate to severe pain. No differences were noted between the two anesthetics, and there was no increase in heart rate in either group. In a parallel study, the success rates of pulpal anesthesia—defined by two consecutive negative readings on the electrical pulp tester obtained within 20 minutes—following intraligamentary injections of 1.4 mL of 2% lidocaine with 1:100,000 epinephrine or 4% articaine with 1:100,000 were similar and ranged from 74% to 86% (Berlin et al). Supplemental intraligamentary injection of 2% lidocaine with 1:100,000 epinephrine in teeth with irreversible pulpitis was 56% successful for pulpal anesthesia (Nusstein et al 2005).

One of the approaches used to increase the success of pulpal anesthesia in teeth with inflamed pulps is to administer an analgesic prior to injection of the local anesthetic. A number of randomized, double-blind, controlled clinical trials have examined the effect of pretreatment with analgesics such as ibuprofen, indomethacin, and acetaminophen on pulpal anesthesia following administration of IANBs. A meta-analysis of these studies reports that preemptive administration of analgesics in patients diagnosed with irreversible pulpitis increases the chance of success of IANBs (Nagendrababu et al).

Variations in methodology and design of the clinical trials may account for the differences in the study results. For example, the methods of determining pulpal anesthesia varied across the studies. Another confounding factor is how the diagnosis of irreversible pulpitis was made. In some studies, the diagnosis was based on the presence of spontaneous pain and a prolonged response to cold, whereas in others it was based only on the cold response. In addition, the response to cold was not well defined in most studies. The periapical status of the symptomatic teeth and duration of symptoms were also not factored into the data analyses. While both peripheral and central mechanisms are thought to contribute to odontogenic pain, it is likely that the central mechanisms play a greater role in patients with symptomatic periapical periodontitis or those who have experienced odontogenic pain for a longer duration than in those with normal periapical tissues or with more acute onset of symptoms.

### Drugs that block central mechanisms of pain perception and hyperalgesia

Opioids are potent analgesics, often used in dentistry in combination with acetaminophen, aspirin, or ibuprofen. Most clinically available opioids activate the μ-opioid receptor. Although opioids are effective as analgesics for moderate to severe pain, their usage is generally limited by their adverse side-effect profile. Opioids induce numerous side effects, including nausea, emesis, dizziness, and drowsiness, and have the potential to cause respiratory depression and constipation. Chronic usage is associated with the development of tolerance and dependence. Because opioid dosing is limited by this side-effect profile, opioids are almost always used in combination with other drugs for the management of dental pain. A combination formulation is preferred because it permits a lower dose of the opioid to reduce side effects.

Pentazocine, a mixed agonist-antagonist opioid, has been shown to be effective in the management of orofacial pain. It acts as a κ-opioid receptor agonist and a partial δ-opioid receptor agonist with mixed agonist-antagonist

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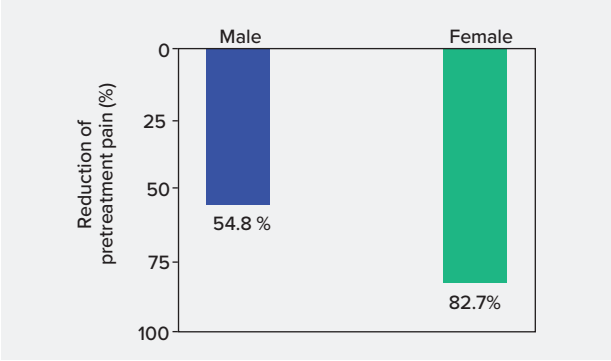
properties at the μ-opioid receptor. In a postendodontic study comparing the analgesic efficacy of a pentazocine (50 mg)–naloxone (0.5 mg) combination (Talwin, Sanofi) to that of ibuprofen (600 mg), female patients who took pentazocine-naloxone reported significantly greater pain relief than did male patients who took the same medication (Ryan et al) (Fig 9-15). Pentazocine-naloxone could be an effective analgesic in female endodontic patients who are unable to take other analgesics such as ibuprofen and acetaminophen. Compared to other opioids, it offers the additional advantage of relatively less potential for opioid dependence and abuse (Swift and Hargreaves).

The gabapentinoids pregabalin and gabapentin, first developed as anticonvulsant drugs, are now prescribed for the treatment of neuropathic pain and chronic pain. Gabapentin is a structural homolog of the neuroinhibitor GABA (gamma-aminobutyric acid) and binds to the α2δ subunit of the voltage-gated calcium channel, thus regulating neurotransmitter release (Hu et al 2018). There is limited evidence on the effectiveness of these drugs for acute pain conditions, including odontogenic pain (Narita et al). In a preclinical model of pulpitis, pregabalin reduced medullary release of the neurotransmitter glutamate and the electromyographic activity induced by mustard oil application to the tooth pulp (Narita et al). In a clinical trial, preoperative administration of 600 mg of gabapentin effectively reduced pain for up to 24 hours after endodontic treatment (Isik et al). It is important to note that gabapentinoids have no anti-inflammatory effect and that they are most effective in the presence of central sensitization. These drugs must be used with caution as evidence suggests that they possess potential for abuse, especially in patients with a history of opioid abuse (Evoy et al).




▲ **FIG 9-15** Sex difference in the analgesic effect of pentazocine-naloxone in postoperative endodontic patients. Patients used a visual analog scale (VAS) to report pain. The reduction in pain was calculated for each patient based on the following formula: ([pretreatment VAS score – postoperative VAS score] / pretreatment VAS score) × 100 (at each time point and averaged over the 24-hour period). (Data from Ryan et al.)

## Pain-Management Strategies

When managing pain in an individual patient, the skilled clinician must customize the treatment plan to the patient, balancing knowledge of pulp biology with general principles of endodontics and restorative dentistry, mechanisms of hyperalgesia, pain-management strategies, and the individual's particular factors (eg, medical history, concurrent medications, etc). The following discussion reviews general considerations for pain-management strategies.

Effective management of endodontic pain starts with the three Ds: diagnosis, definitive dental treatment, and drugs (see Box 9-1). As described earlier in this chapter, the management of endodontic pain should focus on removal of peripheral mechanisms of hyperalgesia, which generally requires treatment that removes or reduces etiologic factors (eg, bacterial and immunologic factors) and nerve terminals. Pulpotomy and pulpectomy represent rational and effective treatments for initial removal of these factors (see Fig 9-8). However, pharmacotherapy is often required to interrupt continued nociceptor input (eg, NSAIDs, local anesthetics) and to suppress central hyperalgesia (eg, opioids).

Administration of NSAIDs alone is usually sufficient for most patients who can tolerate this drug class because of its effectiveness in managing inflammatory pulpal pain and the relatively low incidence of postendodontic pain (Pak and White). Thus, an NSAID prescription such as ibuprofen 600 mg taken every 6 hours is optimal treatment for the majority of patients who can tolerate this drug class. For patients who cannot tolerate an NSAID, a 1,000-mg dosage of acetaminophen is often suitable for managing posttreatment pain.

About 20% of patients may have moderate to severe postendodontic pain, and some patients may not be adequately relieved by a single-drug approach (Pak and White). In general, a flexible analgesic prescription strategy offers the best combination of pain relief and minimal side effects while providing the clinician a rational approach for customizing an analgesic plan to a particular patient's needs (Fig 9-16). A flexible prescription plan serves to minimize both postoperative pain and side effects.

With this goal in mind, the strategy is first to achieve a maximally effective dose of the nonnarcotic analgesic (either an NSAID or acetaminophen for patients who cannot take NSAIDs). Second, in those rare cases when the patient still experiences moderate to severe pain, the clinician should consider adding drugs that increase the analgesia provided by the NSAID (or acetaminophen). Given its predictive value regarding the need for more comprehensive pharmacotherapy, the presence of preoperative hyperalgesia may serve as an indication for considering these NSAID combinations.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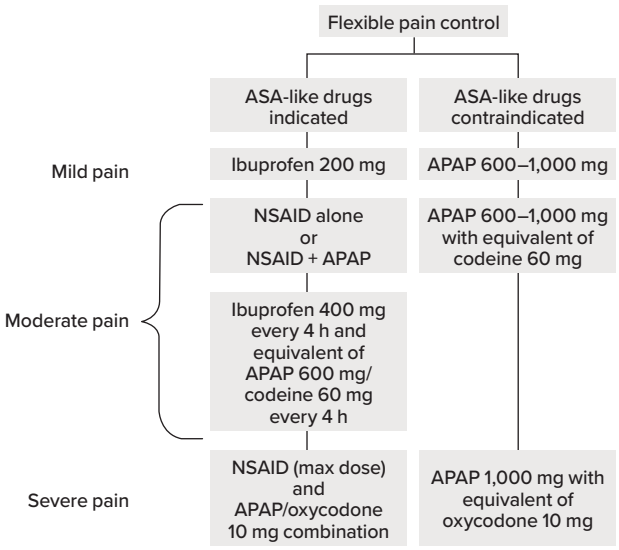
Flexible pain control
ASA-like drugs indicated
ASA-like drugs contraindicated
Mild pain
Ibuprofen 200 mg
APAP 600–1,000 mg
NSAID alone or NSAID + APAP
APAP 600–1,000 mg with equivalent of codeine 60 mg
Moderate pain
Ibuprofen 400 mg every 4 h and equivalent of APAP 600 mg/ codeine 60 mg every 4 h
Severe pain
NSAID (max dose) and APAP/oxycodone 10 mg combination
APAP 1,000 mg with equivalent of oxycodone 10 mg

▲ **FIG 9-16** Flexible prescription plan for management of postendodontic pain based on the patient's ability to tolerate NSAIDs and designed to minimize pain and exposure to side effects. APAP = acetaminophen; ASA = acetylsalicylic acid.

There are two general analgesic approaches for such patients. One approach is to coprescribe an NSAID with acetaminophen. This strategy provides predictable control for severe pain and can be used to modify a standard NSAID-only regimen if pain control is inadequate (Cooper). The concurrent and short-term administration of acetaminophen and an NSAID appears to be well tolerated in most patients without any apparent increase in side effects or alterations in pharmacokinetics (Cooper, Breivik et al, Moore and Hersh). A second general approach is to coprescribe an NSAID with an acetaminophen-opioid combination. All three drugs (the NSAID, the acetaminophen, and the opioid) are active analgesics that can have additive effects when combined (Cooper, Breivik et al, Moore and Hersh).

## Conclusion

The information and recommendations provided in this chapter were selected to aid the clinician in the management of pulpal pain caused by dentinal hypersensitivity or pulpitis and related periradicular pain. However, clinical judgment must also take into account other sources of information, including the patient's history, concurrent medications, nature of the pain, and the comprehensive treatment plan to design the best pain-management program for an individual patient. Integration of these general principles of pain mechanisms and management with the clinician's assessment of each patient's needs is an effective approach for the successful management of pulpal pain.

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# 10

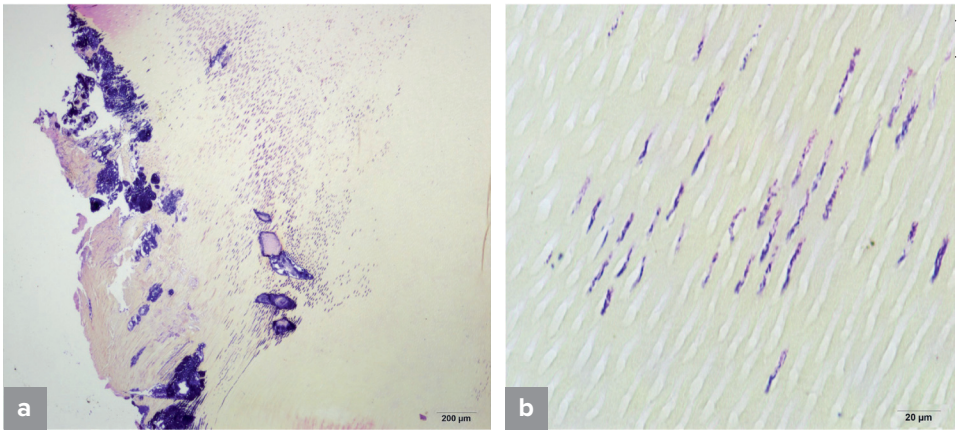
# CARIES, RESTORATIVE DENTISTRY, AND THE PULP

Francisco Wanderley Garcia de Paula-Silva
Carlos Alberto de Souza Costa
Jorge Perdigão
Katherine Divine

Dental caries is a major public health issue that is impacted by the presence of specific microorganisms from the oral microbiome as well as conditions such as excessive sugar consumption and poor dental hygiene (Zhan). This dynamic disease is characterized by the recurring demineralization of tooth structure, which eventually leads to its clinical manifestation (Zero et al, Simon-Soro and Mira).

Enamel surfaces are covered by complex dental biofilms composed of several species of microorganisms (Takahashi and Nyvad). Among these species, the gram-positive *Streptococcus mutans* (*S mutans*) is significantly cariogenic (Zhan). *S mutans* provides an environment rich in extracellular polysaccharides (EPS) and with low pH, which is favorable to acidogenic and aciduric species (Lemos et al). This catabolic process is powered by the conversion of sugar to acid, resulting in the destructive and progressive activity of saccharolytic bacteria on the mineralized tooth structures.

Enamel loss can expose the dentin-pulp complex to opportunistic pathogens of the oral environment. If the dental pulp is not treated, it will initiate an immunoinflammatory response to prevent the spread of bacteria and their toxins to the remaining pulp and periapical tissues (Goldberg et al 2015, Shah et al). The connective tissue in the dental pulp can undergo reorganization and repair through the formation of new mineralized tissue if the noxious agent is eliminated and the tooth is restored. On the other hand, dental pulp inflammation and necrosis will develop if the stimulus persists without treatment or if it is extremely severe due to a high bacterial load or high microbial virulence (Goldberg et al 2006, Goldberg et al 2015, Ribeiro-Santos et al). The contamination of the dental pulp by microorganisms and byproducts arising from caries lesions (Fig 10-1) sustains a persistent immunoinflammatory response, with the production of cytokines and proteolytic enzymes within dental pulp (Hahn and Liewehr 2007a, Hahn and Liewehr 2007b, Hahn and




▶ **FIG 10-1** *(a)* Dental caries penetration in dentin. Bar represents 200 µm. *(b)* Note the presence of gram-positive bacteria within the dentinal tubules. Bar represents 20 µm. (Brown and Brenn bright-field staining.)

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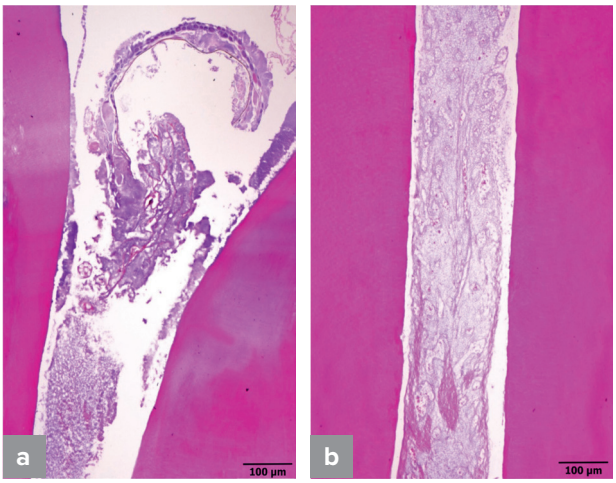
Liewehr 2007c, Paula-Silva et al 2010a). When infection spreads in an apical direction, it culminates in the development of apical periodontitis (Márton and Kiss, Paula-Silva et al 2016, Paula-Silva et al 2020, Petean et al).

## Effects of Caries on Dentin and Pulp

The response of the dental pulp to caries can occur even before microorganisms invade this connective tissue. The first morphologic changes are seen in the odontoblastic layer, which experiences a reduction in both the number and size of odontoblasts (Zero et al). The dentin-pulp complex responds to irritant stimuli through a complex inflammatory response. Several types of immune cells react to the presence of microorganisms through recognition of the pathogen by resident odontoblasts, fibroblasts, dental pulp stem cells, or specialized inflammatory cells (Farges et al 2009, Soden et al, Farges et al 2015). The ability of the immune system to manage the balance between pulpitis and repair is crucial to maintain pulp vitality (Cooper et al).

Initially, the pulpal response is characterized by deposition of intratubular dentin (dentin sclerosis), which partially obliterates the dentinal tubules and causes a reduction in dentin permeability (Goldberg et al 2008). The persistence of pulpal irritants results in increased vascularization, including vasodilation and proliferation of small vessels. As the caries lesion progresses deeper into the dentin, neutrophils and other inflammatory cells are recruited to the area from the surrounding blood vessels. This neutrophil recruitment increases the release of lysosomal enzymes, which aid in the digestion of bacteria. However, these enzymes also contribute to the degradation and dissolution of pulp tissue, resulting in liquefactive necrosis (Fig 10-2). The accumulation of neutrophils leads to the formation of microabscesses and the manifestation of clinical symptoms (Ricucci et al 2014, Ricucci et al 2020).

In deep caries lesions, gram-negative proteolytic anaerobic microorganisms tend to predominate and adapt to the changing environment by using proteins and glycoproteins derived from demineralized dentin, collagen, and dentinal fluids (Paddick et al, Hahn and Liewehr 2007a, Lima et al). This use of alternative nutrient sources empowers bacteria to reach deeper areas of dentin. Interestingly, the dental caries microbiome can generate toxic byproducts including ammonia, urea, and indole, which affect the patient's sensitivity to heat and the presence of painful symptoms (Hahn et al 1993, Khabbaz et al).




▲ **FIG 10-2** *(a)* Dental pulp necrosis due to a caries lesion in the coronal portion of the root canal space. *(b)* The middle third of the tooth root still presents a vital pulp. (Hematoxylin-eosin [h&e] brightfield staining; bar represents 100 µm.)

## Responses of the Dentin-Pulp Complex to Caries

### Immune response

Microorganisms arising from caries lesions cause an inflammatory response in dental pulp that involves cellular and molecular components (Álvares et al). Neutrophils, macrophages, and lymphocytes, among other cells, are recruited to the site of inflammation by local release of cytokines, chemokines, growth factors, and extracellular matrix components in the body's effort to eliminate the injury (Sloan and Smith, Paula-Silva et al 2009, Márton and Kiss, da Rosa et al, Giraud et al).

Different cell types from innate and adaptive immunity participate in host response to dental caries. Cells of the innate immune system have receptors that recognize pathogen-associated molecular patterns (PAMPs), which include bacterial cell wall components such as lipoteichoic acid (LTA) and lipopolysaccharide (LPS) (Pääkkönen et al). These pattern-recognition receptors identify the presence of pathogens mostly through toll-like receptors (TLRs) on the cell surface and leucine-rich nucleotide receptors in the cytoplasm. Interaction of LTA, a structure present in the cell wall of gram-positive bacteria, with TLR2 promotes the nuclear translocation of the transcription factor nuclear factor κB (NFκB) and generates the production of chemokines and cytokines and the recruitment of immature dendritic cells (Farges et al 2009).

Antigens derived from *S mutans* can diffuse into the dental pulp through the dentinal tubules and induce synthesis of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interferon-γ (IFN-γ), which mediates an immune response by dendritic cells and macrophages (Hahn et al 2000, Hahn et al 2004). LTA derived from *S mutans* signaling through TLR2 induces tumor necrosis factor α (TNF-α) and nitric oxide (NO) synthesis in macrophages (Hong et al). Interaction of LPS, found in gram-negative bacteria, with TLR4 and CD14 receptors increases the expression of several proinflammatory cytokines. Nucleotide-binding oligomerization domain 2 (NOD2), an intracellular receptor, recognizes peptidoglycans present in phagocytized gram-positive and gram-negative bacteria and activates mitogen-activated protein kinase (MAPK) and NFκB signaling pathways to generate proinflammatory cytokines (Staquet et al). Direct exposure of the dental pulp to LPS results in the early recruitment of neutrophils and a reduced number of mononuclear cells, which culminates in dental pulp necrosis (Ribeiro-Santos et al) and the development of apical periodontitis (Petean et al).

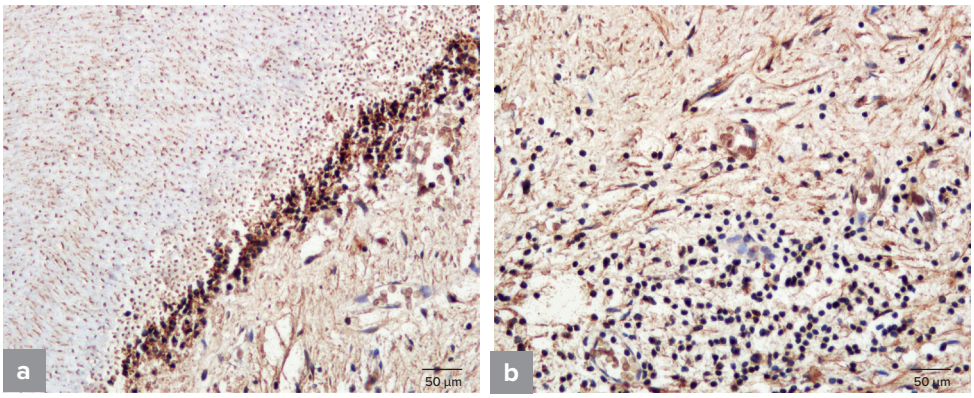
Macrophages are phagocytic cells of innate immunity and, together with neutrophils, provide the first line of defense against dental pulp infection (Iwasaki et al). In addition to their phagocytic function, macrophages act as antigen presenting cells to lymphocytes and take part in the release of proinflammatory cytokines (Bertasso et al). T and B lymphocytes are recruited later on, as the adaptive immune response takes place. Although the role of inflammatory cells in the response to shallow dental caries is poorly understood, in deep lesions various patterns of inflammatory response have been demonstrated (Izumi et al, Hahn et al 2004, Meyle et al).

Biochemical mediators are released locally in order to stimulate the cellular and humoral immune response. Among these inflammatory mediators are eicosanoids, which are synthesized from the metabolization of arachidonic acid. Through the action of cyclooxygenase (COX) or lipoxygenase (LO) enzymes, structural modifications occur in the arachidonic acid chain, leading to the synthesis of prostaglandins and thromboxanes or leukotrienes and lipoxins, respectively (Clària, Lorencetti-Silva et al 2019, da Silva et al 2022).

Leukotrienes have important biologic functions, including chemotactic action, aggregation and degranulation of polymorphonuclear cells, and stimulation of the adhesion of leukocytes to the endothelial wall for the transmigration of inflammatory cells (Rådmark and Samuelsson, Thomas and Puleo, Lorencetti-Silva et al 2022a). Levels of prostaglandin $E_2$ ($PGE_2$) are markedly elevated in inflamed dental pulps with carious exposure (Nakanishi et al, Petrini et al). $PGE_2$ controls the vascular permeability of the dental pulp (Okiji et al) and induces several proinflammatory effects, including vascular dilation, pain, and leukocyte recruitment (Ricciotti and FitzGerald).

TNF-α is a pleiotropic cytokine also produced by the human dental pulp in response to infection or injury (Fig 10-3), and NO is an early-phase mediator of pulpal inflammation responsible for the recruitment of inflammatory cells to the dental pulp (Kawashima et al, Horst et al). Previously, it was demonstrated that *S mutans* induced the synthesis of TNF-α, NO, and $PGE_2$ through NFκB signaling when internalized by macrophages via phagocytosis (Sorgi et al).

Matrix metalloproteinases (MMPs) are enzymes present in the dentin matrix and dental pulp that are involved in tissue remodeling (Mazzoni et al, Paula-Silva et al 2010a). MMPs are characterized according to their substrate specificity and include collagenases (MMP-1, -8, and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10, and -11), matrilysins (MMP-7 and -26), membrane-type MMPs (MMP-14, -15, -16, -17, -24, and -25), and others (MMP-12, -20, and -27). High levels of MMP-9 were found in teeth with pulpitis (Tsai et al), whereas MMP-2 levels were reduced (Gusman et al). Acids are capable of activating MMPs entrapped in dentin matrix during tooth formation, which have collagenolytic and gelatinolytic activities (Queiroz et al 2019). Additionally, dental pulp cells stimulated with TNF-α expressed MMP-1, MMP-2, and MMP-13, demonstrating their importance during proinflammatory events (Paula-Silva et al 2009).




▶ **FIG 10-3** TNF-α is a pleiotropic protein synthesized by resident odontoblasts and dental pulp stem cells *(a)* as well as by inflammatory cells *(b)* recruited to neutralize bacteria arising from dental caries. (DAB [3,3'-diaminobenzidine] immunohistochemistry bright-field staining; bar represents 50 µm.)

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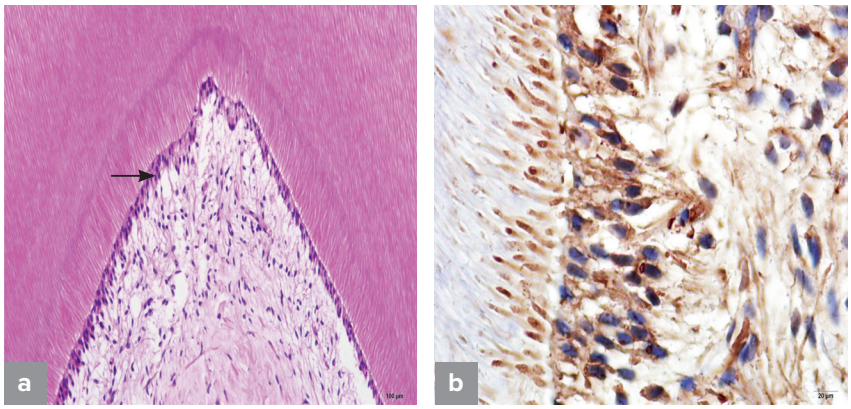
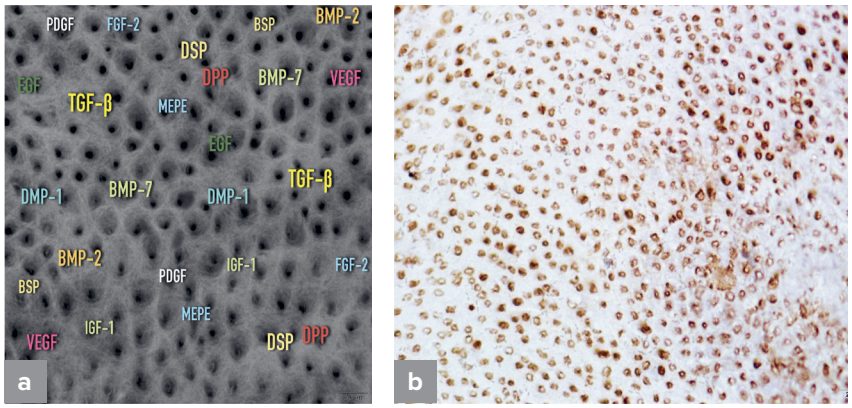
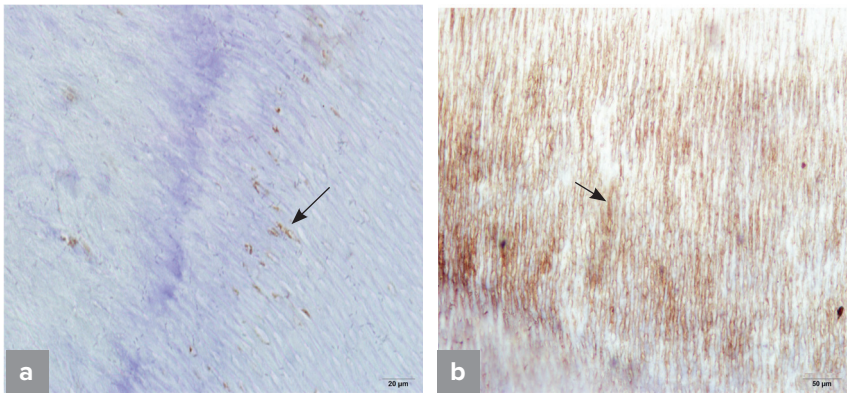



◀ **FIG 10-4** *(a)* Odontoblast cells *(arrow)* have a secretory role and are responsible for the formation of dentin. Note the line that indicates primary and secondary dentin synthesis (h&e brightfield staining). *(b)* Odontoblasts and dental pulp cells synthesize osteopontin (OPN), an important mediator of biomineralization (DAB immunohistochemistry brightfield staining).




◀ **FIG 10-5** *(a)* Numerous bioactive molecules can be found in the dentin matrix. *(b)* Note the presence of dentin matrix protein-1 in the peritubular dentin (DAB immunohistochemistry brightfield staining).




◀ **FIG 10-6** Presence of osteocalcin, a biomineralization molecule *(a, arrow)*, and substance P, a mediator of pain *(b, arrow)*, inside the dentinal tubules. Movement of bioactive molecules within the tubules stimulates odontoblast and dental pulp cell activity. (DAB immunohistochemistry brightfield staining.)

## Reparative responses

Although odontoblasts have an immunocompetent role (Couve et al), their main function is secretory. They are responsible for the formation of primary dentin during tooth development and subsequently for the production of secondary and tertiary dentin (Simon et al). During the progression of a caries lesion, proteins entrapped in the dentin matrix are dissolved and released into the dentinal tubules (Smith et al 2012). These bioactive molecules include growth factors, especially transforming growth factor β (TGF-β), dentin matrix proteins, chemokines, cytokines, MMPs, and other bioactive proteins (Smith et al 2016). These molecules modulate several processes critical to repair, including chemotaxis, angiogenesis, mineralization, mesenchymal stem cell recruitment, and neurogenesis (Figs 10-4 and 10-5).

Dentin sclerosis is one of the first reparative responses following microbial injury and involves the movement of interstitial fluid within the dentinal tubules. The liquid allows the movement of bioactive molecules released from dentin (Fig 10-6) that trigger signaling pathways via membrane receptors present in odontoblasts and/or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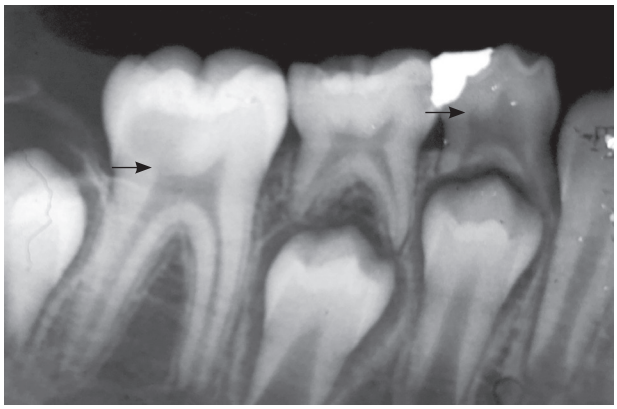
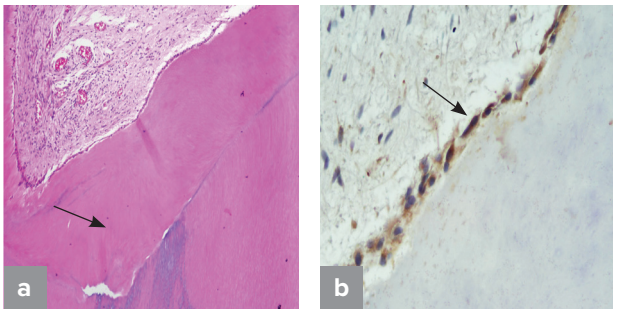


▲ **FIG 10-7** Tertiary dentin formation at the periphery of the pulp tissue adjacent to the caries lesion *(arrows)* in the permanent first molar and primary molar.



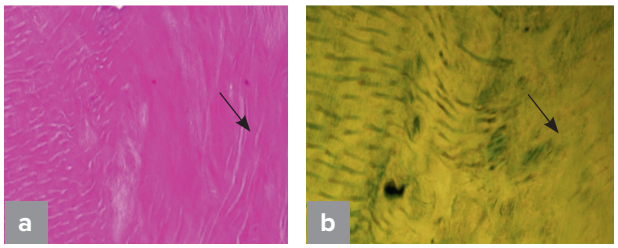

▲ **FIG 10-8** *(a)* Reparative dentin formation *(arrow)* beneath dental caries (h&e brightfield staining). *(b)* Note that the odontoblastic layer has disappeared and novel dental pulp stem cells have been recruited and differentiated toward an odontoblastic phenotype—ie, cells that produce dentin sialoprotein *(arrow)* (DAB immunohistochemistry brightfield staining).

in their cytoplasmic processes. Movement of ions from the inner portion of the dental pulp toward the dentin promotes precipitation of proteins and the deposition of minerals inside the tubules.

Biomediators released from dentin also stimulate the production of proteins important for the synthesis of dentin organic matrix by the odontoblastic layer (Smith et al 2016). This process is known as *tertiary dentin deposition* (Fig 10-7) and takes place at the periphery of the pulp tissue, adjacent to the source of stimulus. Tertiary dentinogenesis comprises a wide spectrum of responses ranging from the secretion of a tubular dentin, which is similar to primary and secondary dentin, to the deposition of an amorphous and atubular dentin, similar to an osteoid tissue (Fig 10-8). The morphology of the tissue that is formed is dependent on the cell type involved in dentin matrix deposition and is labeled either reactionary or reparative dentin (Cooper et al, da Rosa et al, Tziafas).

If the inflammatory process generated is of low intensity, the primary odontoblasts are stimulated to deposit and mineralize a tubular dentin matrix to synthesize reactionary tertiary dentin (Smith et al 2016, Queiroz et al 2019). When aggression is of high intensity and results in death (apoptosis or autolysis) of odontoblasts, dental pulp stem cells are recruited to the region and differentiate into odontoblast-like cells (see Fig 10-8b). These cells begin to secrete the organic matrix of dentin, often amorphous and atubular and sometimes with cellular inclusions in its structure, which is known as *reparative dentin* (Fig 10-9) (Goldberg et al 2008, Daltoé et al).

Dental pulp stem cells are important players in reparative response because they are immunoregulators and capable of self-renewal, multilineage differentiation, and production of biomineralized structures (Li et al, Lorencetti-Silva et al 2022b). Therefore, the dental pulp is an environment with broad capacity for repair, especially because it is composed of progenitor cells that are prone to respond to signaling molecules (Chmilewsky et al). Reparative tissue is mainly composed of type 1 collagen and noncollagenous proteins such as dentin sialophosphoprotein, alkaline phosphatase, dentin matrix protein-1, bone sialoprotein, and osteopontin (Jiao et al, Liu et al, Daltoé et al, Kim et al).




▲ **FIG 10-9** *(a and b)* Reparative atubular dentin formation *(arrows)*. Note the changes in the direction of the dentinal tubules from primary and secondary dentin. (*a*, h&e brightfield staining; *b*, h&e fluorescence staining.)

## Pulpal Injury and Healing Responses to Cavity Preparation

Restorative dentists seek to repair the health of a tooth and its pulp by removing caries and placing compatible restorations; however, each time the tooth is treated, pulpal damage may occur (Abou-Rass). Prior to initiating comprehensive restorative dentistry, the idea of a stressed pulp must be recognized, because repeated insults will result in pulp degeneration.

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While the size of the pulp chamber and depth of caries are individual factors, there are procedural measures and techniques that restorative dentists need to control to reduce further pulpal inflammation. In 1962, Zach and Cohen evaluated how high- and low-speed rotary instruments, with and without cooling devices, raised pulpal temperature. Without water cooling, high-speed and low-speed handpieces raised tooth temperature 11°F and 14°F, respectively. The use of an effective water coolant during high-speed preparation is therefore advised to avoid increasing the pulpal temperature (Zach and Cohen 1965). It should be ensured that this water spray reaches the area of preparation and is in sufficient quantity to continuously wash and refrigerate the area (Langeland and Langeland). Pulp chamber temperature can also be increased by the use of blunt burs and excessive pressure (Ottl and Lauer, Chua et al). Furthermore, it is recommended that when inspecting the cavity or crown preparation, prolonged air drying should be avoided to prevent desiccation and displacement of the odontoblastic layer into the dentinal tubules. Previous research by Cotton indicated that air drying displaced odontoblast nuclei into dentinal tubules within 30 seconds, thereby disrupting the odontoblastic layer.

Dentinal permeability can also be affected by operative procedures. The use of an acid etch during the placement of restorations removes the dentinal smear layer created by the cavity preparation and opens dentinal tubules, allowing bacterial penetration (Bergenholtz 1991). Michelich et al found that within 5 days, bacteria can grow through or be filtered by pressure through acid-etched dentin, whereas unetched dentin facilitates fluid filtration but prevents bacterial penetration. Therefore, efforts should be made to limit dentinal permeability in order to reduce or eliminate the chance for bacterial infiltration.

Laser-assisted tissue ablation is an option for carious tissue removal and cavity preparation. Due to the presence of water and hydroxyapatite crystals in the composition of both enamel and dentin, the laser's emitted energy can be absorbed by both structures when exposed to light, with a wavelength comparable to the water absorption peak (Corona et al, Chan et al). However, when a high-intensity laser is applied to the tooth surface, the water rapidly changes from liquid to gas by absorbing the laser's electromagnetic energy (Meister et al). This procedure dramatically increases the internal pressure of the mineral substrate, therefore breaking its structure through the ablation process. The temperature of the irradiated tissue rises as a result of this process (Contente et al, Yilanci et al).

## Remaining Dentin Thickness

In 1966, Reeves and Stanley observed that pulp changes are negligible when the distance between the pulp and bacterial penetration is more than 1.1 mm. When the caries lesion reaches a distance of 0.5 mm or less from the pulp, pathosis begins to develop. Invasion of reparative dentin results in irreversible pathosis (abscess and granulation tissue). Consequently, even small to moderate-sized caries lesions and restorations can cause pulpal irritation. Dentin thickness and tubular permeability are determinants of the pulpal inflammatory response to dental caries (Murray et al).

Pulpal inflammation occurs beneath superficial caries before microorganisms in the carious process have made contact with pulpal tissue. It has also been shown that bacterial components can migrate through the dentinal tubules to trigger inflammatory responses (Bergenholtz 1977, Warfvinge et al). In addition to nonspecifically activating inflammatory responses, bacterial products in carious dentin may potentially behave as antigens capable of interacting with the immune system. In vitro studies have revealed that the thickness of the dentin influences the concentration of the LPS and LTA that finally reach the pulp chamber (Pissiotis and Spångberg, Damen et al). An increase of inflammatory cells or genes encoding important neutrophil proteins in teeth with pulpitis has been observed when caries lesions were located 1.5 to 2.0 mm from the pulp (Izumi et al, McLachlan et al).

## Selective Dental Caries Removal

The use of minimally invasive procedures for removal of carious tissue has increased for both primary and permanent teeth (AAPD 2020, Duncan et al). One of these procedures is the selective removal of the carious tissue, which can be performed in one or two steps (stepwise).

In one-step selective removal of carious tissue, excavation is restricted to the most superficial layer of carious dentin, known as *infected dentin*, which clinically appears as a necrotic and disorganized structure. Infected dentin is rich in microorganisms, toxins, and proteolytic enzymes, and its structure cannot be reorganized or remineralized. Below the infected dentin layer, there is a demineralized structure, with an altered color but a structure that is partially maintained, known as *affected dentin*. The affected dentin also contains microorganisms, although in smaller numbers, and can be remineralized using bioactive dental materials or by a host-mediated process (Innes


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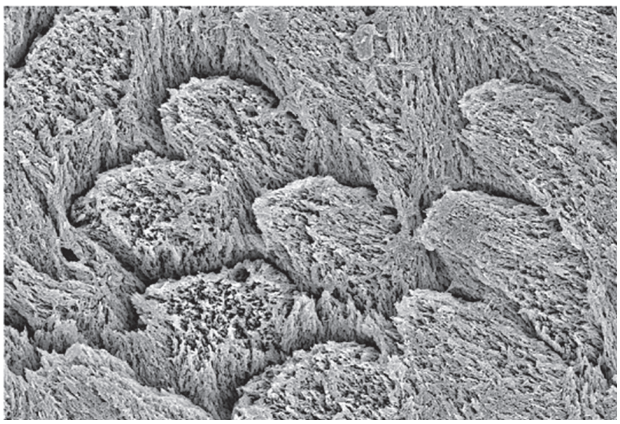
et al). After removal of the infected dentin, it is recommended that a dental material with antimicrobial activity be applied to the affected dentin, so that it can act on any remaining microorganisms, inhibiting their activity and metabolism. This procedure allows the caries process of the deeper layer of dentin to stop, enhancing or creating suitable conditions for the repair of the dentin-pulp complex (Schwendicke et al 2016b).

In the stepwise procedure, an interim restoration is placed following excavation. After 6 to 9 months, a reintervention is necessary to remove the temporary material and place the final restoration (Bjørndal et al). The purpose of this treatment is to avoid exposure of the pulp tissue during caries excavation (Orhan et al, Ricketts et al, Schwendicke et al 2013).

The objective of performing indirect pulp capping (with no clinically visible dental pulp exposure) is to reduce the number of viable residual microorganisms, remineralize dentin demineralized by the acidic microenvironment of the caries lesion, induce tertiary dentin deposition, and isolate the pulp from thermal stimuli (About et al). Calcium hydroxide, mineral trioxide aggregate (MTA), silicate, and glass-ionomer cements are products used in these situations because they are able to inhibit microbial activity and remineralize the dentin affected by dental caries (Bhavana et al, Hashem et al 2015, Ghatole et al, Hashem et al 2019, Maltz et al 2018, Duncan et al). In the presence of dental pulp tissue exposure, it is necessary to apply a protective biomaterial such as calcium hydroxide, MTA, or calcium silicate cements (Hilton et al, Schwendicke et al 2016a, Rosa et al 2018, Paula et al 2018, Stafuzza et al). Several preclinical studies in animal models (da Silva et al 2007, Daltoé et al) and randomized clinical studies (Nowicka et al 2013, Nowicka et al 2015, Cuadros-Fernández et al, Silva et al 2019) have demonstrated the effectiveness of these materials when used as capping agents applied directly or indirectly on the dental pulp.

Although the risk of exposing the pulp tissue is reduced, the composition of the microbiota associated with the degree of disorganization of the pulp tissue may interfere with the production of mineralized tissue by the pulp tissue (Ricucci et al 2020). Reduction of the cariogenic substrate via removal of the largest microbial contingent present in the infected dentin may slow down the progression rate of the caries lesion (Maltz et al 2007, Alves et al). Nonetheless, long-term randomized clinical trials should be conducted to assess the effects of the remaining bacteria and their metabolites on the pulp tissue at a subclinical level (histologic and molecular) (Schwendicke et al 2016b).



▲ **FIG 10-10** Scanning electron micrograph (SEM) of enamel etched with 35% phosphoric acid for 15 seconds (original magnification ×5,000).

## Dentin Bonding with Resin-Based Materials

In 1955, Buonocore reported the use of phosphoric acid to "render the enamel surface more receptive to adhesion in the same manner as it does for metals." This enamel acid-etch technique is still the gold standard for bonding resin-based materials to tooth structure. The micromechanical interaction of adhesives with enamel is the result of the diffusion and interlocking of resin monomers into the microporosities left by the acid dissolution of enamel (Fig 10-10). Enamel is an ideal substrate for bonding because it contains 90 vol% of hydroxyapatite without vital structures. These characteristics make enamel compatible with the hydrophobic nature of resin-based restorative materials.

In 1952, Kramer and McLean published the results of an in situ study in teeth scheduled to be extracted for orthodontic reasons. After the application of a chemically cured adhesive (Sevitron) to dentin, they observed a "narrow zone of material staining deeply with haematoxylin immediately bordering the cavity" under the optical microscope. This was the first reference to the structure currently known as the dentin *hybrid layer*, which is paramount to dentin adhesion (Nakabayashi et al). While bonding to enamel is a simple clinical procedure based on the enamel acid-etch technique, bonding to dentin presents a much greater challenge. Dentin is intrinsically humid (hydrophilic), crossed by a dense network of fluid-filled tubules that radiate outward from the pulp. Dentin contains approximately 45 vol% of hydroxyapatite and 33 vol% of organic extracellular matrix, the remainder being water. Approximately 10 vol% of the matrix is composed of a fibrous net of type 1 collagen along with

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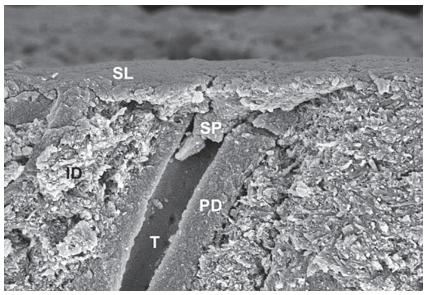
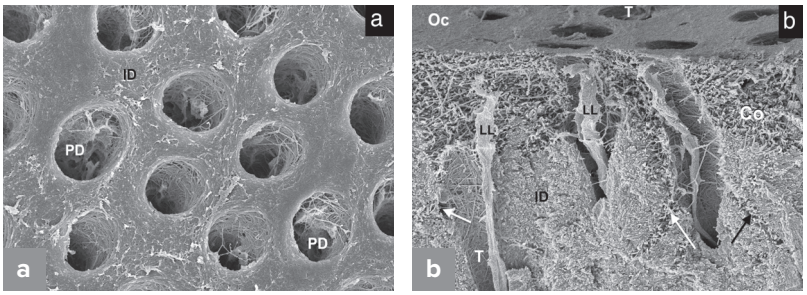



▲ **FIG 10-11** SEM of dentin smear layer created with a carbide bur (original magnification ×10,000). SL = smear layer; SP = smear plug; ID = intertubular dentin; PD = peritubular dentin; T = tubule.




▲ **FIG 10-12** *(a)* SEM depicting an occlusal view of dentin etched with 30% phosphoric acid for 15 seconds. *(b)* SEM depicting a lateral view of dentin etched with 32% phosphoric acid for 15 seconds. (Original magnification ×5,000.) ID = intertubular dentin; PD = peritubular dentin; T = tubule; Oc = occlusal surface; Co = collagen fibers; LL = lamina limitans; *arrows* = tubular anastomoses.

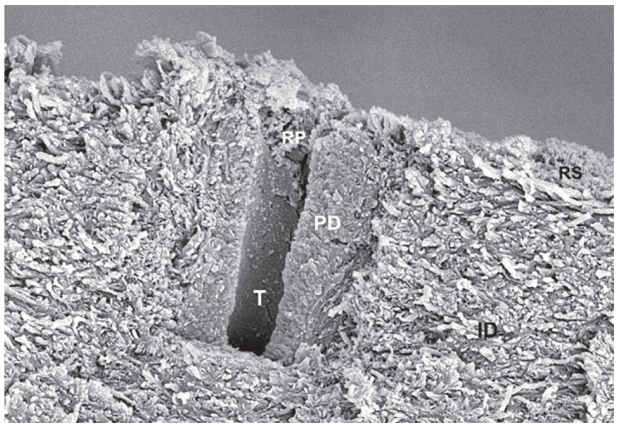
minor amounts of noncollagenous proteins including phosphoproteins, phospholipids, enzymes, proteoglycans, and serum proteins (Garberoglio and Brännström, Tjäderhane et al). As opposed to enamel, the humid and organic nature of dentin is not compatible with durable bonding.

## Current Dentin Adhesives

The dentin smear layer contains residual organic and inorganic components (Bowen et al). It is created when dentin is instrumented with a bur or an excavator. The debris obliterates the dentinal tubules, forming "smear plugs," which decrease dentin permeability by nearly 90% (Pashley et al) (Fig 10-11).

Dentin adhesives are categorized using two different classifications. The classification from 1st to 8th generation is often used by the dental industry to underline the latest trends (Perdigão). However, it misses the components of the dentin adhesive system (ie, etchant, primer, and bonding resin), making it confusing for clinicians. The classification by adhesion strategy is more informative, whereby dentin adhesives are grouped according to their interaction with the smear layer, as follows:

- Etch-and-rinse (ER) adhesives include 30% to 40% phosphoric acid as a separate etching step prior to the application of the adhesive. Traditional phosphoric acid etchants dissolve the smear layer, open the tubules, and demineralize dentin to a depth of 2 to 7 µm (Fig 10-12).
- Self-etch (SE) adhesives do not use a separate etching step. They do not remove the smear layer but incorporate it into the adhesive interface (Fig 10-13).




▲ **FIG 10-13** SEM depicting a lateral view of dentin treated with an SE primer (original magnification ×10,000). RS = smear layer infiltrated by the resin in the primer; RP = resin-infiltrated smear plug; ID = intertubular dentin; PD = peritubular dentin; T = tubule.

### Etch-and-rinse adhesives

Removal of the smear layer with etchants led to improvements in bond strength (Kanca, Van Meerbeek et al 1994). The simultaneous application an acid to enamel and dentin is known as the *total-etch technique* or the *etch-and-rinse technique*. The demineralization depth depends on the composition of the acidic gel, application time, and concentration (Perdigão et al 1996). The newest generation of less viscous phosphoric acid gels are less aggressive to dentin than their predecessors (Perdigão) without reducing dentin bond strengths. The dentin demineralization depth is less than 2.5 µm for these etchants (Perdigão).

Once the etching gel is rinsed off, the hydrophilic primer of three-step ER adhesives is applied on the dentin surface and air dried to evaporate the solvents, followed by the application of a hydrophobic bonding resin that requires light curing. Two-step ER adhesives combine the primer and the hydrophobic bonding resin into one solution.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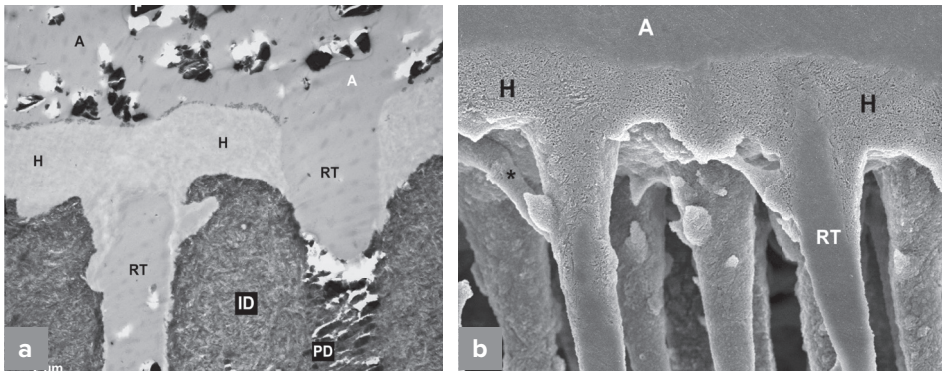


▶ **FIG 10-14** *(a)* Transmission electron micrograph (TEM) depicting the interface between an ER adhesive and dentin (original magnification ×4,000). *(b)* SEM depicting the interface between an ER adhesive and dentin. Dentin was dissolved to uncover the interface morphology (original magnification ×5,000). A = adhesive; H = hybrid layer; RT = resin tag; * = intertubular anastomosis; ID = intertubular dentin; PD = peritubular dentin.

Because of this simplification, more than one coat of the combined adhesive solution of two-step ER adhesives is needed to achieve acceptable micromechanical interlocking of monomers into dentin (Perdigão). Three-step ER adhesives result in higher dentin bond strengths and better clinical performance than two-step ER adhesives (Armstrong et al, Peumans et al 2014). When ER adhesives are applied on etched dentin, they fill the spaces between exposed collagen fibers (see Figs 10-12b and 10-14), where they polymerize to form a hybrid tissue of collagen wrapped in resin from the adhesive known as the *hybrid layer* (Fig 10-14).

The concept of moist dentin (or wet bonding) prior to the application of ER adhesives used to be very popular because low bond strengths were obtained when dentin was air dried. However, leaving the dentin moist may not be so critical with current adhesives because they contain water, which may result in residual water being left behind after evaporation of the solvents and subsequent degradation of the resin-dentin interface. More crucial than leaving dentin moist is the agitation of the adhesive during application, which improves the permeation of the resin monomers into etched dentin even if dentin is slightly dried. A clinical study found that passive application of the adhesive resulted in an 82.5% retention rate after 2 years compared to a 92.5% retention rate of the restorations when the adhesive was scrubbed vigorously (Loguercio et al).

Demineralization of dentin to a depth greater than that to which the adhesive can infiltrate and envelop the collagen fibers results in lower bond strengths (Hashimoto et al 2002). The area of the hybrid layer in which the collagen fibers are not surrounded and protected by the resin is susceptible to degradation (Van Meerbeek et al 1994). Overetching is not an issue when dentin etching time is increased from 15 to 30 seconds (Perdigão and Lopes), but it may occur when dentin is etched more than once, a technique that has been taught in dental schools in case of blood or saliva contamination of the dentin surface.

## Self-etch adhesives

Self-etch (SE) adhesives are less aggressive and more user-friendly than ER adhesives, as their application time is reduced. SE adhesives do not require a separate acid-etching step because they condition and prime enamel and dentin simultaneously due to the acidity of the phosphate resin monomer in their primer. However, the enamel bond strengths are lower than those of ER adhesives. Etching enamel separately without etching dentin, also known as the *selective enamel etching technique*, improves the bond strength of SE adhesives to enamel. A systematic review of clinical trials reported that selective enamel etching resulted in less marginal discoloration, better marginal integrity, and increased retention of composite restorations bonded with SE adhesives (Szesz et al).

SE adhesives are available as one-step and two-step adhesives. Two-step SE adhesives include an acid primer (usually a phosphate resin monomer) and a separate hydrophobic bonding resin similar to the hydrophobic bonding resin in three-step ER adhesives. One-step SE adhesives combine the acidic primer and the hydrophobic bonding resin into one solution. Water is always incorporated in the primer solution of SE adhesives because it is needed to ionize the phosphate monomer and trigger its interaction with enamel and dentin. Because dentin is not etched with phosphoric acid, SE adhesives infiltrate through smear layers (Fig 10-15; see also Fig 10-13) coupled with partial dissolution of hydroxyapatite to generate a hybrid layer with smear layer remnants and residual hydroxyapatite crystallites (Van Meerbeek et al 2003b).

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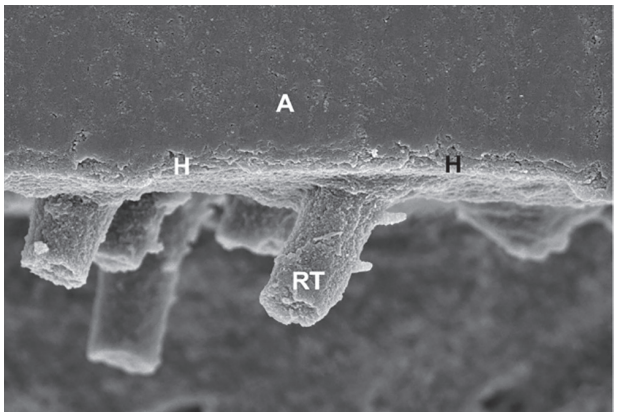
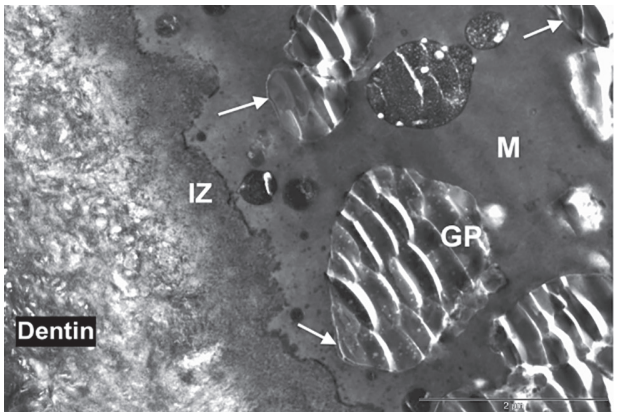



▲ **FIG 10-15** SEM depicting the interface between an SE adhesive and dentin (original magnification ×10,000). A = adhesive; H = hybrid layer; RT = resin tag.




▲ **FIG 10-16** TEM depicting the interface between a GIC and dentin (original magnification ×20,000). IZ = interaction zone; GP = glass particle; M = polycarboxylate matrix; *arrows* = silica gel surrounding the glass particle.

As opposed to one-step SE adhesives, two-step SE adhesives have the potential to seal dentin and form a stable hybrid layer (Perdigão et al 2008) as a result of the application of the hydrophobic bonding resin over the acidic primer. The acidic monomer 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) included in the primer of two-step SE adhesives provides stable chemical bonding to $Ca^{2+}$ ions in the hybrid layer, which has resulted in excellent clinical retention of the mild (pH > 1.5) SE adhesive Clearfil SE Bond (Kuraray) (Peumans et al 2015) at 13 years. In addition, clinical studies have reported that mild SE adhesives result in more reliable dentin bonding than more acidic SE adhesives (pH < 1.5) (Peumans et al 2014).

## Self-adhesive materials

Self-adhesive materials combine dentin adhesive and restorative components into the same material.

### Composite resins

Clinical trials have demonstrated that self-adhesive composite resins do not result in acceptable outcomes (Poitevin et al, Çelik et al). These materials are not indicated for endodontics.

### Traditional glass-ionomer cements

Glass-ionomer cements (GICs) have been used as self-adhesive materials for over 50 years. The first version of a GIC was introduced by Wilson and Kent in 1971. The cement presented a nonshrinking setting reaction, chemical adhesion to tooth structure, fluoride release, antibacterial activity, and biocompatibility with pulp tissue (Sasanaluckit et al). The adhesion results from the reaction between aluminosilicate glass and a polycarboxylic acid, usually polyacrylic acid. GICs borrow their self-adhesive properties from zinc polycarboxylate cement, as they share the same polycarboxylic acid as the liquid component. The major advantage of all GICs is their stable chemical bonding and sealing of dentin margins, which makes them excellent materials as intraorifice barriers (John et al) and coronal restorations with margins in dentin (Peumans et al 2014). Their bonding mechanism involves carboxylate ions of the GIC that replace phosphate ions of the dentin substrate to establish stable ionic bonds with $Ca^{2+}$ ions of hydroxyapatite (Yoshida et al), slowly forming a dynamic ion-exchange layer between the tooth and the GIC (Ngo et al, Ngo). This is simultaneously an ionic bonding mechanism and a self-etching interdiffusion nanomechanical interaction, also known as the *surface interaction zone* or *surface intermediate layer* (Fig 10-16). This bonding mechanism is believed to be one of the reasons for the clinical success of GICs (Tay et al 2004, Ngo).

Traditional GICs do not need to be activated with light or with a chemical initiator because they do not contain resin. They set via a spontaneous acid-base reaction.

### Resin-modified GICs

The first resin-modified GIC (RMGIC) was introduced in the late 1980s as Vitrabond, later Vitrebond (3M) (Antonucci et al, Mitra). RMGICs incorporate resin monomers such as 2-hydroxyethyl methacrylate (2-HEMA) into the traditional GIC structure as well as chemical initiators and/or photoinitiators, improving the mechanical properties of traditional GICs and allowing better control of the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setting reaction. Therefore, the setting reaction of RMGICs is identical to the acid-base reaction in traditional GICs but is supplemented with a resin polymerization reaction as in composite resins. The resin component of RMGICs may cause slight polymerization contraction. RMGICs are easier to use than their traditional counterparts, mechanically stronger, and more esthetic. In addition, they are less susceptible to initial moisture contamination.

RMGICs provide stable ionic bonding similar to traditional GICs. The methacrylated polycarboxylic acid present in RMGICs bonds chemically to $Ca^{2+}$ in hydroxyapatite. This chemical bond is a significant factor in the excellent long-term adhesion of RMGICs (Mitra et al) and their clinical outcomes (Sidhu).

The advantages of traditional GICs and RMGICs over composite resins are biocompatibility and a coefficient of thermal expansion similar to that of tooth structure, which together make GICs ideal materials for dentin replacement in wide tooth preparations. Their antibacterial activity stems from their low initial pH and subsequent fluoride ion release. The fluoride release is initially high but declines to a lower and sustained level over several years. However, GICs are rechargeable with topical fluoride applications to increase their fluoride release (Diaz-Arnold et al).

Systematic reviews have reported enough evidence for GICs (both traditional and resin-modified) to be considered the best dentin adhesive/restorative material in noncarious cervical lesions (Peumans et al 2005, Peumans et al 2014). RMGICs have also been successfully used as dentin adhesives underneath composite resin restorations (Tyas and Burrow, Takahashi et al). While several recently introduced materials have marketing claims of being RMGICs, they are not considered true GICs because they do not set through an acid-base reaction (McLean et al, Perdigão). Their setting reaction is initiated with light polymerization.

## Limitations of Dentin Bonding

In general, the major shortcoming of resin-based dentin adhesives in endodontics is their potential for bacterial leakage when the cavosurface margin is in dentin/cementum or in enamel that has not been etched with phosphoric acid. Other limitations include the following:

- Dentin adhesion is not reliable as the source of primary retention in clinical situations without enamel margins or without enough residual tooth structure, such as in composite core build-up restorations (Zenthöfer et al).
- Postoperative sensitivity in posterior adhesive restorations is related to composite polymerization shrinkage stresses that cause cusp deflection (Lee and Park, Ferracane and Hilton).
- Monomers often used in dentin adhesives, such as HEMA, may impair the protective immune response in odontoblasts and pulp fibroblasts (Schweikl et al).
- Dentin adhesives cause pulpal inflammation if applied in deep dentin (Costa et al).
- The water in the composition of SE adhesives, including recent universal adhesives, may become entrapped in the resin-dentin interface after polymerization if not properly evaporated. Residual water results in nanoleakage (Luque-Martinez et al).
- The acidity of one-step SE adhesives inhibits the polymerization of chemically cured composites. An extra dual-cure activator is mixed with simplified SE adhesives to make them compatible with dual-cured composite build-up materials and self- or dual-cured luting cements (Tay et al 2003).
- One-step SE adhesives behave as permeable membranes after polymerization, allowing the permeation of fluids through the adhesive layer to the surface and subsequent degradation of the resin-dentin interface by hydrolysis (Tay et al 2002).

## Response of Dental Pulp to Dental Materials

The development of new biomaterials that can replace tooth structure has been the focus of researchers and manufacturers for many years. As a result, there are currently numerous dental materials with varying chemical compositions, properties, and indications. It is important to carefully consider the biocompatibility of these dental materials as well as their mechanical and physical properties. In many cases, the materials are applied directly to the pulp tissue or may interact with the dental pulp indirectly. If a material does not fulfill the biologic recommendations for clinical usage, it can cause a chronic inflammatory reaction and disorganization of the pulp tissue, which will lead to necrosis. To ensure the safe use of new restorative materials, it is essential to use a range of well-established in vitro and in vivo methodologies to assess their cytotoxic effects and biocompatibility as well as to establish safer protocols for their application.

## Response of Dental Pulp to Bleaching Agents

In-office tooth bleaching is considered a noninvasive procedure that results in rapid chromatic change of hard dental tissues. However, postbleaching dentinal sensitivity seems to be the main side effect reported by patients subjected to this treatment (Kielbassa et al, Maran et al, Meireles et al). Although the discomfort caused by tooth sensitivity

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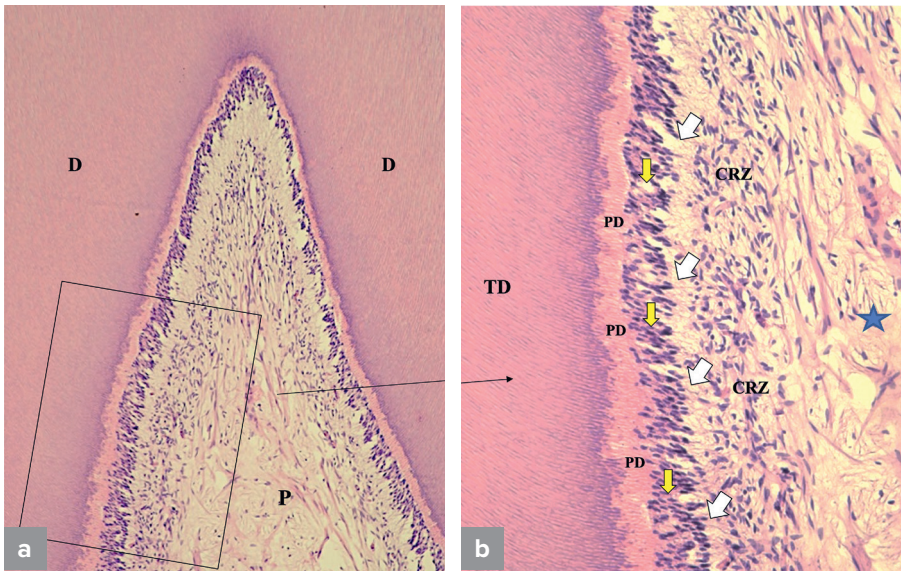


◀ **FIG 10-17** *(a)* Pulp horn of a sound human tooth. Note that the odontoblastic layer is underlying the thin layer of predentin. The central zone of the pulp exhibits small blood vessels and fibroblasts among a well-defined collagen-rich extracellular matrix (h&e staining; original magnification ×64). D = dentin; P = pulp. *(b)* Detail of the area delimited in *a*. The following layers that characterize the dentin-pulp complex can be clearly observed: tubular dentin (TD), homogeneous predentin (PD), continuous odontoblastic layer *(yellow arrows)*, cell-free zone *(white arrows)*, cell-rich zone (CRZ), and central zone of the pulp *(blue star)* (h&e staining; original magnification ×125).

is transitory, a number of patients have described this posttreatment pain as an unpleasant experience that can disrupt treatment continuity (Soares et al 2014, Kose et al). Researchers have suggested that bleaching sensitivity is related to the ability of hydrogen peroxide ($H_2O_2$) to diffuse in high concentrations through the enamel and dentin, causing damage to dental pulp cells (Soares et al 2014). Several investigations have shown that a portion of the $H_2O_2$ generated by bleaching treatments that does not interact with the chromophores present in enamel and dentin rapidly migrates toward the pulp chamber. The transamelodentinal diffusion of these toxic reactive species is also time dependent (Ubaldini et al, Cavalli et al, Kury et al).

Higher $H_2O_2$ concentrations in contact with pulp cells are associated with severe cellular damage, according to laboratory research (Soares et al 2015a, Soares et al 2019, Ortecho-Zuta et al 2021). Additionally, randomized clinical investigations revealed that postbleaching discomfort appears to be limited to anterior teeth (de Almeida et al, Bonafé et al) (Fig 10-17). This is because the amount of $H_2O_2$ that reaches the pulp chamber is proportional to the enamel/dentin thickness of bleached teeth. In this way, small teeth, such as mandibular incisors, are more susceptible to the cytotoxic effects of $H_2O_2$ released from bleaching gels.

Histopathologic studies performed in human teeth demonstrated that in-office bleaching with highly concentrated gels (> 35% $H_2O_2$) caused severe damage to the pulp of mandibular incisors. The changes were characterized by deep tissue disorganization, areas of necrosis, and local inflammation (Roderjan et al 2014, Roderjan et al 2015). These deleterious pulpal responses caused by tooth bleaching agents were observed in 100% and 60% of incisors of young and elderly patients, respectively. On the other hand, premolars subjected to the same bleaching treatment did not exhibit such intense adverse pulpal effects (Kina et al, de Souza Costa et al 2010). In view of this, it is contraindicated to use the same high-concentration bleaching protocol for different teeth without taking the enamel and dentin thicknesses into account (Fig 10-18; see also Fig 10-17).

A recent in vitro study revealed that bleaching gels containing concentrations of $H_2O_2$ lower than 10% caused limited toxicity to human dental pulp cells when applied for 15 minutes to enamel/dentin discs simulating the thickness of maxillary central incisors (Soares et al 2015b). Despite this positive result, the experimental low-concentration bleaching gels did not achieve an acceptable esthetic effect. To establish nontoxic and painless in-office teeth bleaching techniques, a series of laboratory and clinical research studies have been completed (Duque et al, Soares et al 2019, Ortecho-Zuta et al 2019). In one of the studies, the addition of chemical activators to gels with 35% $H_2O_2$ that were applied to enamel for 30 to 45 minutes increased the bleaching efficacy and diminished the transamelodentinal diffusion of free $H_2O_2$, which reduced the cytotoxic effect of treatment. Despite the interesting results, the researchers reported that the catalysis of $H_2O_2$ performed by the addition of enzymes and metal oxides still caused significant cell injury.


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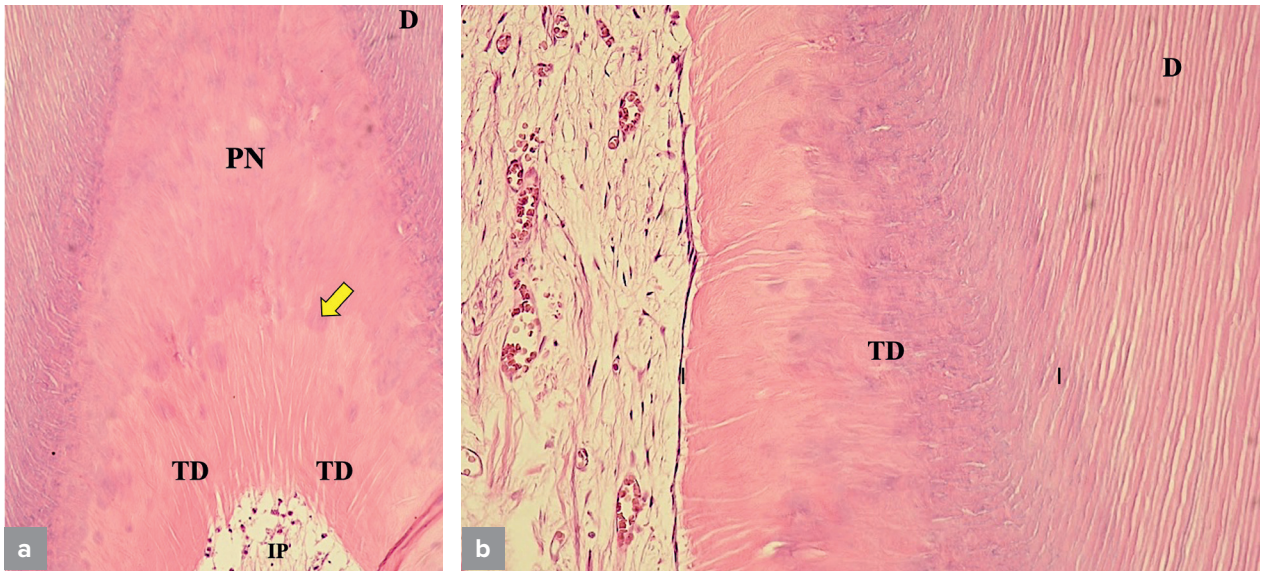


▲ **FIG 10-18** *(a)* Pulp horn of a sound human mandibular incisor subjected to an in-office bleaching procedure whereby a high-concentration gel was applied to the enamel. Partial necrosis of the coronal pulp is observed (PN). Note that a thick layer of tertiary dentin (TD) with mineralized nodules *(yellow arrows)* was deposited below the tissue necrosis. The partially degraded pulp tissue exhibits a discrete inflammatory reaction mediated by mononuclear cells (IP) (h&e staining; original magnification ×96). D = dentin. *(b)* Detail of the radicular pulp of the same bleached mandibular incisor. A thick layer of tertiary dentin (TD) is observed between the tubular dentin (D) and the pulp tissue, which exhibits a number of congested blood vessels (h&e staining; original magnification ×250).

Covering the enamel with polymeric biomaterials (porous nanofiber scaffold and catalyst primer) prior to the application of bleaching gels with high concentrations of $H_2O_2$ may reduce the transamelodentinal cytotoxicity produced by traditional in-office dental bleaching (Soares et al 2019, Ortecho-Zuta et al 2019, Ortecho-Zuta et al 2021). This is because the porous scaffold may reduce the amount of $H_2O_2$ capable of reaching the adjacent enamel/dentin hard tissues. Additionally, the organic portion of the scaffold and the catalytic agent added to the polymeric primer may cause intense degradation of the $H_2O_2$, generating other reactive molecules with higher oxidizing power and shorter lifetime. This method has the ability to prevent pulp cell damage and enhance the bleaching efficiency of treatment (Ortecho-Zuta et al 2021).

In order to sustain the results established through the clinical usage of gels containing high concentrations of $H_2O_2$, researchers assessed a commercial gel containing 35% $H_2O_2$ that was applied for 15 or 30 minutes to uncoated enamel or enamel previously coated with experimental polymeric biomaterials (Ortecho-Zuta et al 2022). These procedures were done over the course of three successive sessions with the intent of evaluating the esthetic effect of repeated treatments and correlating them with transamelodentinal cytotoxicity levels. Covering the enamel with a porous scaffold and an enzyme-loaded primer enabled the esthetic efficacy promoted by conventional in-office dental bleaching to be achieved in only 15 minutes of application of gel with 35% $H_2O_2$ on enamel, which also significantly diminished the cytotoxicity (Ortecho-Zuta et al 2022).

Furthermore, taking into consideration the excellent results obtained by catalyzing $H_2O_2$ and increasing the production of molecules with high oxidation potential, researchers evaluated the association of using polymeric biomaterials and photocatalyzing bleaching gels with 10%, 20%, and 35% $H_2O_2$ (Martins et al 2022). Coating of the enamel with a nanofiber scaffold and a polymeric catalyst primer, followed by the application of bleaching gels irradiated with violet LED light, stimulated $H_2O_2$ degradation, increased esthetic efficacy, and significantly reduced the cytotoxicity of the treatment, according to the authors' findings (Fig 10-19).

Using a 10% $H_2O_2$ bleaching gel in this combined catalysis strategy optimized the esthetic result and restricted the

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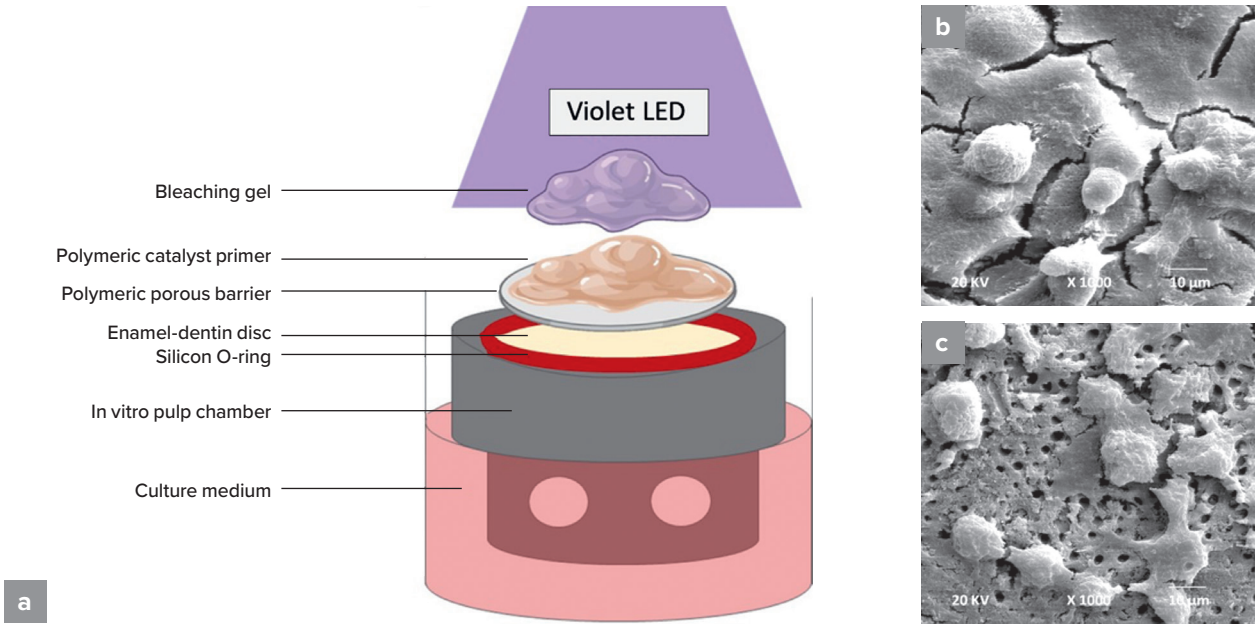


▲ **FIG 10-19** *(a)* Representation of the in vitro methodology widely employed to assess the esthetic efficacy, degradation kinetics, and transamelodentinal cytotoxicity of bleaching gels and new strategies recommended for in-office tooth bleaching. Note that a standardized enamel-dentin disc is adapted to an in vitro pulp chamber. Silicon O-rings and wax are used to prevent unadvertised diffusion of toxic components released from the bleaching gel. Cells are seeded on the dentin surface of the enamel-dentin disc. In this specific experimental condition, after covering enamel with the biopolymers (catalyst primer and porous barrier), the bleaching gel is applied and then irradiated with violet LED light. Cells that remain attached to dentin are evaluated concerning their viability and morphology. Bleaching gel components that diffuse through the disc reach the culture medium (called *extract*), which is collected and applied to pulp cells seeded in well plates for complementary analysis of cytotoxicity. The amount of $H_2O_2$ present in the extract is also determined. *(b)* Morphology of the MDPC-23 cells seeded on the pulp surface of the dentin disc. Note that the cells are confluent and no dentinal tubules are exposed. (SEM; original magnification ×1,000.) *(c)* A high amount of $H_2O_2$ reached the MDPC-23 cells seeded on the dentin surface. Note that only a small number of cells with deep morphology changes remained attached to the substrate. A large area of tubular dentin can be observed. (SEM; original magnification ×1,000.)

transamelodentinal toxicity of the treatment. Therefore, one can anticipate that the combination of these strategies will contribute to the success of future professional tooth bleaching, resulting in satisfactory esthetic outcomes without causing pulpal damage or patient discomfort.

## Response of Dental Pulp to Resin Cements

In clinical practice, resin luting cements with or without bonding agents have been widely utilized. These dental materials possess important properties, including enhanced esthetics and adhesion to enamel and dentin. On the other hand, their potential to cause pulp tissue toxicity is a significant concern. This negative effect is directly attributable to the presence of unreacted free monomers released by resin-based materials. These monomers can induce pulp cell death mediated by oxidative stress and overexpression of inflammatory mediators. The depletion of glutathione peroxidase and superoxide dismutase enzymes by these materials has also been reported (Hanks et al, Stanislawski et al, Lefeuvre et al, Chang et al 2012, Krifka et al). Additionally, unreacted free monomers can change the phenotypic characteristics of dental pulp stem cells, which may interfere with the healing process of the dentin-pulp complex (Bakopoulou et al, Galler et al, Martins et al 2012, Paschalidis et al).

The morphology and permeability of the dentin is intimately related to the pulp, and it is therefore important for dentists to be aware of the potential adverse effects caused by specific cementation protocols in restorative dentistry and how to prevent them. Some clinical scenarios will inevitably expose deep dentinal layers, mainly during tooth preparation. It is known that the diameter and number of dentinal tubules per area increases during tooth preparation. Dentinal fluids can interfere with the polymerization of resin-based materials applied to the surface of the preparation due to the high permeability of the dentinal substrate (Abedin et al, Paschalidis et al). In addition, the chemical composition, handling, and placement technique of dental materials can also influence their compatibility (de Souza Costa et al 2002a, de Souza Costa et al 2006, Tjäderhane).


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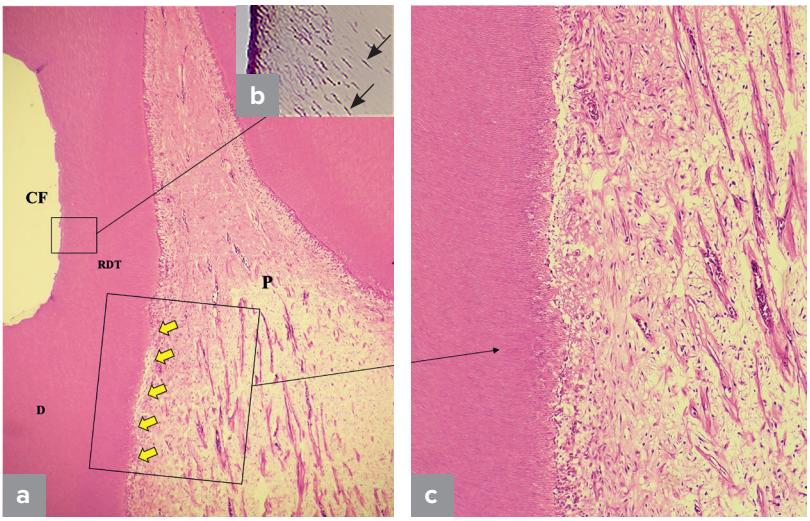


▶ **FIG 10-20** *(a)* Deep class V cavity prepared in a human premolar. Very thin remaining dentin thickness (RDT) is observed between the cavity floor (CF) and the pulp tissue (P). Note the intense disruption of the odontoblastic layer related to the cavity floor *(yellow arrows)* (h&e staining; original magnification ×32). *(b)* Representative image of the area delimited in *a*. Long resin tags are present inside the dentinal tubules. Note the intense transdentinal diffusion of resin components toward the pulp *(arrows)* (Brown & Brenn staining; original magnification ×250). *(c)* High magnification of the area delimited in *a*. Note the complete disruption of the odontoblastic layer and intense disorganization of the subjacent pulp layers. A moderate inflammatory reaction mediated by mononuclear cells and a number of small blood vessels can be observed (h&e staining; original magnification ×125).

Laboratory and clinical investigations carried out in the last decades showed that the procedure of conditioning the dentin substrate with acid agents exposes a network of collagen fibers and demineralizes the peritubular dentin. In these studies, the authors demonstrated that removing the smear layer and enlarging the dentinal tubules prior to the application of adhesive systems increased the cytotoxic potential of restorative procedures. This adverse effect was exacerbated by the substrate's high moisture content (de Souza Costa et al 2006, de Souza Costa et al 2007, Tjäderhane). Several studies have demonstrated that the application of dental materials with a high HEMA content on permeable dentin results in intense indirect toxic effects to pulp cells (de Souza Costa et al 2002a, Lanza et al). This occurs because HEMA has a low molecular weight and a high hydrophilicity, which facilitates its transdentinal diffusion toward pulp tissue (Gerzina and Hume 1995, Gerzina and Hume 1996, Hamid and Hume, Barbosa et al) (Fig 10-20). Besides causing intense oxidative stress on pulp cells, free resin monomers also reduce the expression of type 1 collagen, osteonectin, and dentin sialoprotein, which play a central role in the process of dentin matrix deposition and mineralization (Imazato et al). Therefore, on a permeable dentin substrate, HEMA-free dental materials that do not need dentin etching prior to clinical usage may provide a more biocompatible and safer solution (Cetingüc et al).

To understand the influence of the association of adhesive systems and resin luting cements on the transdentinal cytotoxicity of cementation procedures, Soares et al (2016a) performed an experiment whereby two HEMA-free resin cements were applied to thin dentin discs (0.3 mm). The self-adhesive RelyX Unicem (3M) was applied directly to the dentin substrate, and RelyX ARC (3M) was applied to dentin previously conditioned and then hybridized with an adhesive system. RelyX Unicem caused a discrete reduction in cell viability (lower than 20%) and a slight reduction in the total protein (TP) production and alkaline phosphatase (ALP) activity. On the other hand, cell viability, TP production, and ALP activity were significantly increased when RelyX ARC was used. Although RelyX Unicem and RelyX ARC do not contain HEMA in their composition, it is known that these materials have other toxic molecules, such as triethylene glycol dimethacrylate (TEGDMA) and bisphenol A-diglycidyl ether methacrylate (Bis-GMA), which are capable of affecting the metabolism of dental pulp cells (Soares et al 2016a). The positive results observed for RelyX Unicem may be attributed to the presence of methacrylate monomers with acidic groups in its composition, which eliminates the necessity of prior hybridization of the dentin using an ER adhesive system. Therefore, the protocol advising the clinical use of RelyX Unicem does not substantially enhance dentin permeability, thereby avoiding or at least lowering the transdentinal diffusion of unreacted toxic monomers at high concentrations (Radovic et al, Ülker et al). The size and hydrophilicity of free monomers produced from resin-based materials, which have been identified as deciding variables for their diffusion over dentin, are also of interest (Eliades et al, Makkar and Malhotra). Despite presenting a diffusion rate lower than that of HEMA, TEGDMA is also capable of diffusing through dentinal tubules, which indicates that only a small amount of TEGDMA and other components released from RelyX Unicem probably diffused across dentin, causing only a slight reduction of cell viability (Cetingüc et al).

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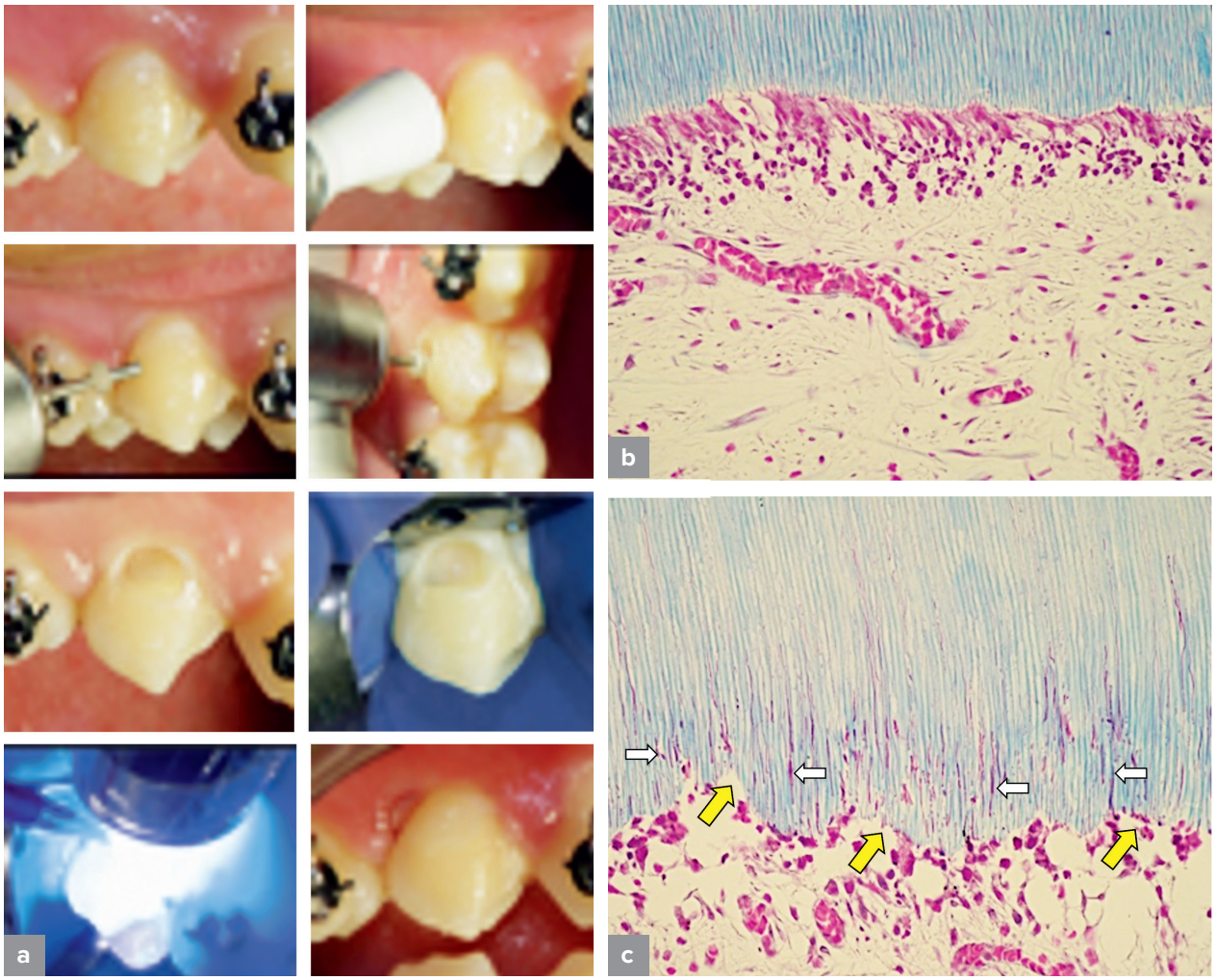
Considering the pertinent scientific data gathered from in vitro and in vivo research, a variety of self-adhesive resin-based luting cements were recommended with the primary goal of lowering the number of clinical steps and enhancing biocompatibility with the dentin-pulp complex (Radovic et al, Ülker et al). In a histopathologic study performed in human premolars (de Souza Costa et al 2006), the pulp tissue was evaluated following cementation of inlays in deep cavities using the resin cements RelyX Unicem and Variolink II (Ivoclar). For Variolink II, the ER adhesive system Excite (Ivoclar) was applied to the dentin before using the resin luting cement, as recommended by the manufacturer. Variolink II was discovered to cause displacement of resin components across dentinal tubules and persistent pulpal inflammation. The intensity of the inflammation and tissue disorganization was inversely related to the remaining dentin thickness between the cavity floor and the pulp. In this study, the authors revealed that the hybridization of dentin using an ER adhesive technique prior to the application of resin luting cement (the standard protocol) might cause damage to pulp cells (de Souza Costa et al 2006). RelyX Unicem does not require prior conditioning and hybridization of dentin with adhesive systems. This resin-based cement is chemically initiated by the presence of potassium persulfate, which reduces the number of residual free monomers (Bourke et al, Kakaboura et al, Abedin et al) (Fig 10-21).

Because of the reactions between carbon bonds of acidic methacrylate with methacrylate monomers present in RelyX Unicem, the resin matrix of this material after setting features a high degree of cross-linking between and




▲ **FIG 10-21** *(a)* Representative images of a sequence of clinical procedures performed in sound human premolars. The large, deep class V cavities were restored by cementation of inlays using the resin luting cements RelyX Unicem and Variolink II. *(b)* Overall, slight tissue disorganization and mild inflammation were observed in the pulp of those teeth in which RelyX Unicem was used. Note that the odontoblastic layer is preserved (Masson's trichrome staining; original magnification ×125). *(c)* In general, when the cavity walls were conditioned and hybridized with a bonding agent and the inlays were cemented using Variolink II, intense disruption of the odontoblastic layer occurred. A number of pulp cells were aspirated into dentinal tubules *(white arrows).* A moderate inflammatory pulp reaction mediated by mononuclear cells and tissue edema were also observed (Masson's trichrome staining; original magnification ×125).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among monomers. In addition, the high number of fillers in RelyX Unicem minimizes its degradation rate (Kakaboura et al). These silanated fillers become chemically embedded in the cement matrix after setting (Radovic et al, Ülker et al). All of these relevant characteristics of RelyX Unicem confer long-term stability and low toxicity to this dental material.

Furthermore, an in vitro investigation was conducted to evaluate the potential transdentinal cytotoxicity of modern light-cured resin luting cements that were photoactivated through 1 mm of ceramic veneer (Araújo et al). Resin-based luting cements RelyX Veneer (3M), Variolink Esthetic LC (Ivoclar), and Allcem Veneer APS (FGM Dental) were applied to dentin previously acid-conditioned and hybridized, as recommended by the manufacturers. All treatments caused transdentinal toxicity to pulp cells of variable intensity. The most cytotoxic effect was observed for Allcem Veneer APS, which was associated with the adhesive system Ambar Universal (FGM Dental). This adverse effect may be attributed to the application protocol indicated by the manufacturer. While a single coat of Single Bond (3M) and Tetric N-Bond Universal (Ivoclar) was applied to acid-conditioned dentin before application of RelyX Veneer and Variolink Esthetic LC, respectively, two coats of the Ambar Universal system were used to hybridize the dentin substrate prior to the application of Allcem Veneer APS. The toxicity is explained by the amount of unreacted cytotoxic monomers on porous dentin substrate, which increases when more of the adhesive system is applied.

It is known that most light-cured resin luting cements are mainly composed of high–molecular weight monomers that increase their viscosity, such as Bis-GMA, TEGDMA, and urethane dimethacrylate (UDMA) (Kitagawa et al). It was also demonstrated that when applied to wet dentin, resin-based materials may suffer a phenomenon defined as the *separation phase*. In this condition, large and hydrophobic monomers remain at the superficial region of the dentin, and small and hydrophilic monomers are displaced further into the dentinal tubules (Eliades et al, Habib et al, Cadenaro et al). In contrast to the hydrophilic nature and lower molecular weight of HEMA and Bis-GMA, other large monomers have a limited capacity to permeate across dentinal tubules (Ye et al). For this reason, this category of resin-based dental cements exhibits little indirect cytotoxic potential. This fact indicates that rather than the resin luting cements themselves, the transdentinal toxic effects observed after cementation of prosthetic parts would be closely related to the composition of the adhesive system and the application protocols employed to hybridize the dentin substrate.

Researchers have previously suggested that the potential transdentinal cytotoxicity of resin-based materials is inversely proportional to the monomer-to-polymer conversion. While the resin-based cements RelyX Veneer, Variolink Esthetic LC, and Allcem Veneer APS are photoactivated with a high-power LED device for 20 to 40 seconds (800 mW/cm$^2$; VALO), each correlated bonding agent is only light cured for 10 seconds, as recommended by their manufacturers. This short irradiation time does not achieve adequate polymerization for bonding agents (Kerezoudi et al, Bianchi et al, Breschi et al). A longer exposure of the resin luting cement to the curing light may increase the monomer-to-polymer conversion in the adhesive system. This method is expected to decrease the number of unreacted monomers available for diffusion through the dentinal tubules, reducing damage to pulp cells (transdentinal toxicity). Most resin-based cements have a substantial indirect toxic effect to odontoblast-like MDPC-23 cells (Kitagawa et al 2022). However, the RelyX Veneer cement coupled with the Single Bond adhesive system is considered the least aggressive cementation protocol. This result could be partially explained by the fact that the bonding agent is light cured for 50 seconds and that the RelyX Veneer cement contains the photoinitiator Ivocerin in its composition. This photoinitiator acts as a polymerization booster by presenting a greater light polymerization ability when compared with the usual camphorquinone, and it absorbs light at a higher wavelength range, between 400 and 450 nm (Leprince et al). Photopolymerization time and the photoinitiatior may help to explain the lower transdentinal toxicity caused by the cementation protocol in which RelyX Veneer cement was associated with Single Bond. Kitagawa et al also noticed that the presence of a 1-mm-thick ceramic veneer between the LED device and the dental material had no effect on the transdentinal cytotoxicity generated by the evaluated cementation techniques.

The thickness of the dentin substrate on which the resin-based materials will be applied, the characteristics of the light-curing unit, and the appropriate time for photoactivation are all factors that may intensify or prevent side effects to the dentin-pulp complex.

## Response of Dental Pulp to Adhesive Materials (GICs and Adhesive Systems)

GICs and RMGICs are a group of dental materials based on the acid-base reaction between poly(alkenoic) acid and an ion-leachable silicate glass. In recent decades, a number of laboratory studies showed that most RMGICs have poor biocompatibility and cause severe toxic effects when applied directly to cultured mammalian cells (Oliva

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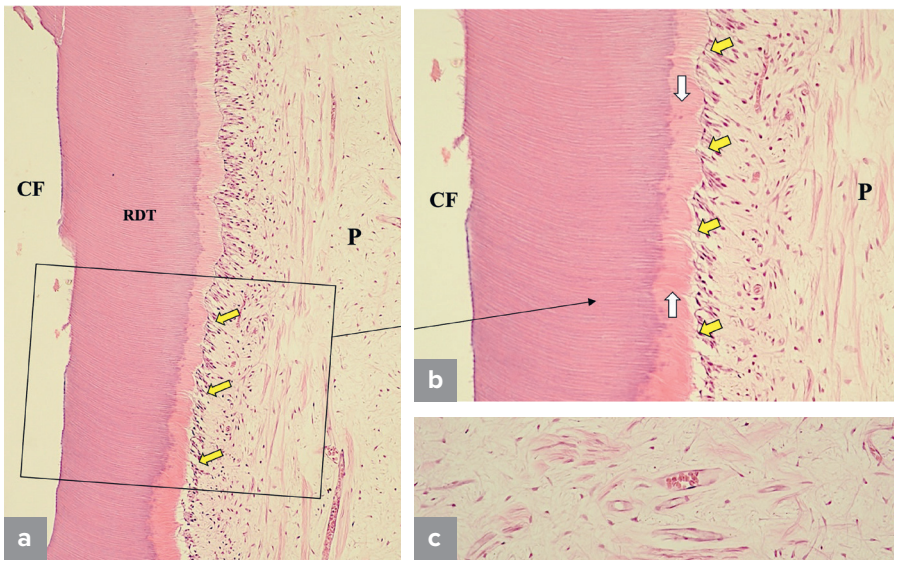


◀ **FIG 10-22** *(a)* Deep class V cavity prepared in a human premolar. The RMGIC was applied directly onto the cavity floor (CF), and the tooth was submitted to adhesive restoration. A very thin remaining dentin thickness (RDT) between the CF and the pulp tissue (P) is observed. Despite the discrete reduction in the number of odontoblasts *(yellow arrows)*, the cell-free and cell-rich zones are preserved (h&e staining; original magnification ×64). *(b)* Representative image of the area delimited in *a*. Homogeneous predentin *(white arrows)* and a continuous layer of odontoblasts *(yellow arrows)* are clearly observed. The subjacent pulp tissue zones do not exhibit signals of inflammatory reaction (h&e staining; original magnification ×125). *(c)* Detail of the central zone with normal histologic features (h&e staining; original magnification ×250).

et al, Geurtsen et al, Mendonça et al 2007, de Souza Costa et al 2003b). However, using dentin discs adapted to in vitro pulp chamber models, researchers demonstrated that a light-cured version of the original GICs may cause only slight or no transdentinal cytotoxicity to pulp cells (Mendonça et al 2012). RMGICs are recognized to be biomimetic materials with similar physical characteristics to dentin. This cement has been shown to continue releasing fluoride ions, to be antimicrobial, and to chemically bond to dentin. Despite the antibacterial activity and biocompatibility of hard-setting calcium hydroxide, this material is highly soluble in a wet environment, presents poor mechanical properties, and does not adhere to dentin substrate. As such, RMGICs were considered as potential materials to replace calcium hydroxide. Currently, RMGICs are advised for usage as a liner/base for definitive restorations and for atraumatic restorative treatment (Frencken, Berg and Croll). However, when the residual dentin thickness between the cavity floor and the pulp is less than 300 µm, a biocompatible liner should be used to preserve pulp vitality (de Souza Costa et al 2014). Considering all the advantages and properties of RMGICs, several in vivo investigations were conducted to determine their biocompatibility.

In an interesting in vivo study performed two decades ago, researchers assessed the response of the pulp tissue against RMGIC Vitrebond or an ER adhesive system (One-Step, Bisco) applied in deep class V cavities prepared in human premolars (de Souza Costa et al 2003a). All teeth were extracted 5 to 30 days after the clinical procedures and then processed for microscopic analysis. Long resin tag formation and diffusion of dental material components through dentinal tubules was observed only when the adhesive system was applied directly to the acid-conditioned dentin substrate. As the remaining dentin thickness decreased, the severity of the inflammation and disorganization of the pulp tissue increased. This may be explained by the fact that the number of dentinal tubules per square millimeter is almost three times higher in deep dentin than in superficial dentin. In addition, the inner diameter of the dentinal tubules close to the pulp (deep dentin) is larger (3.0 µm) compared to the diameter of the tubules in superficial dentin (about 0.8 µm). In this way, the enlargement of the dentinal tubules and smear layer removal caused by deep dentin conditioning allowed an intense and continuous outward dentin fluid movement capable of inhibiting the monomer-to-polymer conversion and interfering with the permeation of the bonding agent among the exposed collagen fibrils. It was previously shown that inward dentin fluid movement that occurs due to heat generated during polymerization may carry uncured toxic monomers across dentinal tubules that can reach the pulp space (Pashley, Hashimoto et al 2004). Thus, it is suggested that the inward dentin fluid movement triggered by light curing of the adhesive system applied on the wet, acid-conditioned deep cavity floor was responsible for the intense pulpal damage observed in this study, in which residual free monomers reached the odontoblasts. On the other hand, no pulpal damage was observed when the cavity floor was lined with RMGIC before hybridizing the conditioned dentin (Fig 10-22). de Souza Costa et al (2003a) concluded that the application of an adhesive system to acid-conditioned deep dentin is not an appropriate procedure. In this specific clinical condition, the cavity floor should be lined with a biocompatible dental material such as Vitrebond.


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In another study performed in human teeth, researchers evaluated the biocompatibility of the following resin-based materials: Vitrebond, Ultra-Blend Plus (Ultradent), Clearfil SE Bond (Kuraray), and the hard-setting calcium hydroxide cement Dycal (Caulk/Dentsply). These dental materials were applied as liners to nonconditioned dentin of deep cavities prepared in human teeth, as recommended by the manufacturers. All teeth were extracted at 7 days or between 30 and 85 days after concluding the clinical procedures (de Souza Costa et al 2007). The authors showed that all liners present acceptable biocompatibility. However, the bonding agent caused a persistent moderate inflammatory pulpal response when applied in cavities that exhibited minimal remaining dentin thickness (300 μm). In this specific condition, transdentinal diffusion of resin components was observed. Given that under clinical conditions it is difficult to identify the thickness of the residual dentin between the cavity floor and the pulp, it appears acceptable and safe to recommend the application of a biocompatible liner prior to dentin hybridization.

Most in vivo studies performed in the last two decades were conducted with RMGICs that were available in a powder/liquid form. These dental cements present components that are hand mixed immediately before their clinical application. More recently, paste/liquid versions of RMGIC liners were developed and introduced to the dental market in convenient dispensing devices. The main purpose was to facilitate handling by offering an improved mixing ratio and shortening the clinical time (de Souza Costa et al 2011). One of these paste/liquid RMGIC formulations is Vitrebond Plus Light Cure Glass Ionomer Liner/Base (3M), which has been available for clinical use in a proprietary "clicker" device. Using human teeth extracted as part of orthodontic treatment, the biocompatibility of Vitrebond Plus was compared to its conventional powder/liquid formulation (Vitrebond) (de Souza Costa et al 2011). Following application of the liners to the floor of deep cavities, all teeth were submitted to adhesive restoration. After 7 or 30 to 60 days, the teeth were extracted and processed for histologic evaluation. For most of the teeth evaluated, the two RMGICs elicited mild initial pulpal inflammation that decreased over time. No inflammatory pulpal reaction to the cavity lining occurred for either RMGIC in those teeth in which the remaining dentin thickness was close to or greater than 500 μm. It seems that the incorporation of Bis-GMA, a bifunctional monomer more toxic than HEMA, into the paste component of Vitrebond Plus did not significantly increase the pulpal response to this liner. The authors reported that the high molecular weight and low hydrophilicity of Bis-GMA probably reduced its transdentinal diffusibility. However, while both RMGICs were considered biocompatible liners for the specific clinical recommendation of use, Vitrebond Plus showed a tendency to be more toxic to the pulp.

Because the pulp biocompatibility of Vitrebond cannot be extrapolated or expected for other GICs that have different chemical compositions and application protocols, some recent in vivo studies were carried out. In one of these studies, the GICs Riva Light Cure and Riva Self Cure (SDI) were applied in deep cavities prepared in human premolars, and the pulpal response was microscopically assessed at time intervals of 7 or 30 days (Ribeiro et al). In most teeth, both ionomeric materials caused initial mild inflammation and discrete pulp tissue disorganization, which persisted only in a few teeth lined with Riva LC. In view of these results, these two liners could be labeled biocompatible. However, Riva LC clearly presented a tendency to produce a more toxic effect to the pulp tissue than Riva SC. The authors hypothesized that the persistent pulpal inflammation caused by Riva LC was related to the transdentinal diffusion of HEMA to reach the pulp tissue. Both liners tested in this study were applied to dentin previously conditioned with polyacrylic acid for 10 seconds, as recommended by the manufacturer (Ribeiro et al).

Previous studies showed via chromatography tests that HEMA is released from RMGICs immersed in a liquid medium (Geurtsen et al, Hamid et al). This may be correlated with the high water sorption of the liner/base materials containing HEMA, which is promptly released in a wet environment (Kanchanavasita et al, Mendonça et al 2007). In a previous in vitro investigation, researchers demonstrated that the most intense toxic effects to pulp cells caused by light-cured RMGIC in comparison with its self-cure version occur due to the high amount of HEMA released from the resinous GIC (Noorani et al). Considering that the inward dentinal fluid movement is increased when dental materials are light cured (Hashimoto et al 2004), Ribeiro et al suggested that the partial smear layer removal caused by the acidic agent allowed transdentinal diffusion of unreacted HEMA and even camphorquinone to the pulp. This event probably caused the greater pulpal damage when Riva LC was used in comparison with Riva SC, which has no resin monomers in its composition (Ribeiro et al). Based on these data, one may consider that the application of RMGICs to deep dentin conditioned with polyacrylic acid is not safe to maintain pulpal health. In addition to negatively influencing the biocompatibility of the RMGICs, conditioning deep dentin with acidic agents seems to be a clinical step capable of enhancing the solubilization of dental material applied directly to wet dentin substrate. Hence, the continuous release of components from resin-based materials may cause persistent pulpal damage that is more intense when mimimal remaining dentin thickness exists.

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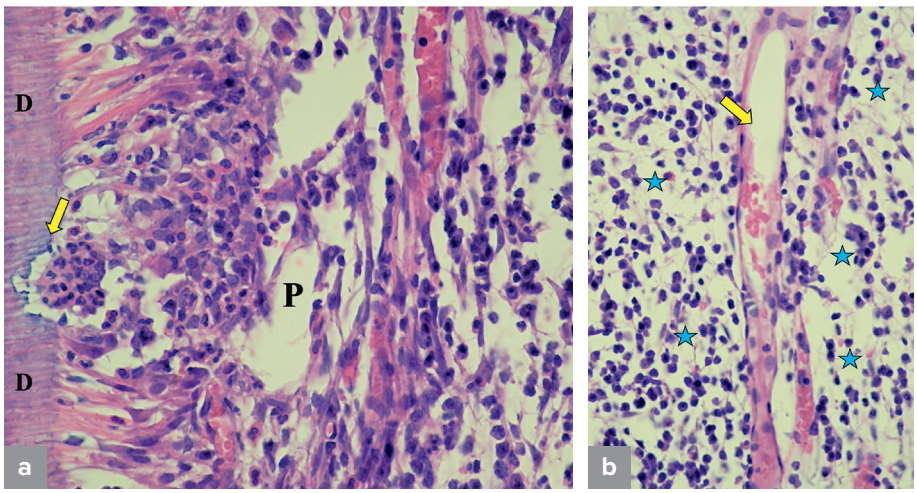


◀ **FIG 10-23** *(a)* Area of pulp tissue related to the floor of a deep class V cavity prepared in a human tooth. After conditioning all cavity walls, a bonding agent was used to hybridize the dentin substrate, and the tooth was restored with composite resin. The odontoblastic layer was totally disrupted, and the predentin was lost. Note the intense mononuclear inflammatory reaction associated with inner dentin resorption *(yellow arrow)* (h&e staining; original magnification ×340). D = dentin; P = pulp. *(b)* Central zone of the pulp of the same tooth presented in *a*. An intense, chronic inflammatory reaction, characterized by notable infiltration of mononuclear cells among a number of congested blood vessels *(yellow arrow)* and associated with collagen degradation and local edema *(blue stars)*, can be observed (h&e staining; original magnification ×250).

It is known that Vitrebond, which has been widely evaluated and is considered the most biocompatible RMGIC available in dentistry, is recommended to be used only as a liner or base product. RMGIC Vitremer (3M), on the other hand, is indicated for direct restoration and core buildup; this ionomeric dental material has been widely employed in pediatric dentistry (de Souza Costa et al 2003b, Mendonça et al 2012). However, the use of Vitremer requires previous application of a light-cured acidic primer (polyacrylic acid + HEMA) to the dentin substrate. Because conditioning deep dentin with acidic agents can increase the cytotoxic effects caused by resin-based materials, the transdentinal toxic effects may be intensified. In order to assess the response of human pulps to Vitremer, this dental material was used as a liner in deep cavities prepared in young human permanent teeth. The pulpal response against Vitremer was compared with that caused by Vitrebond and Dycal (Soares et al 2016b). All cements were applied strictly to the cavity floor, and the teeth were restored with composite resin. Only for Vitremer, the cavity floor was pretreated with the acidic primer, as recommended by the manufacturer. Teeth were extracted after 7 or 30 days and processed for microscopic evaluation. Transdentinal diffusion of resin components associated with disruption of the odontoblastic layer, moderate to intense inflammatory response, and resorption of inner dentin was observed in a number of teeth when Vitremer was used as a liner in cavities that exhibited a remaining dentin thickness of 0.3 mm. This remarkable pulpal damage was caused by the intense transdentinal diffusion of unreacted HEMA released from the primer and even from Vitremer. Despite the high amount of HEMA in the Vitremer composition and the necessity of prior conditioning of dentin substrate with an acidic agent containing HEMA, it seems that the manufacturer of this RMGIC does not recommend lining the cavity floor with a biocompatible dental material before tooth restoration. A similar severe pulpal response has also been observed when acid-conditioned deep dentin of human teeth is hybridized with adhesive systems (de Souza Costa et al 2002a) (Fig 10-23).

On the other hand, no pulpal damage occurred when Vitrebond or Dycal was applied to the cavity floor (Soares et al 2016b). The mild inflammatory pulpal response observed for Vitrebond at the 7-day period was resolved with time. Based on the results presented by Soares et al (2016b) and considering the specific clinical condition in which the dental cements were applied (deep dentin), Vitremer and its protocol of use cannot be considered biocompatible with the pulp tissue. For instance, a biocompatible liner should be applied in deep areas of dentin before proceeding with restoration of the dental cavities with Vitremer.

Based on the excellent results obtained in human teeth following use of Vitrebond as a liner in very deep dentin, this resin-based cement was wrongly recommended


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for direct pulp capping. In addition, despite the intense pulpal damage caused by adhesive systems applied to acid-conditioned deep dentin or even SE adhesives used for the same clinical condition, this category of dental materials was also indicated to be used in pulp capping. The rationale behind the use of RMGICs and adhesive systems instead of recognized capping products based on calcium hydroxide was that the prevention of leakage by chemical/micromechanical interlocking of such resin-based materials to cavity walls is more critical for the pulp healing processes than the compatibility of the capping agent to the pulp. Clinicians, lecturers, and a number of researchers considered that the positive results observed after applying RMGICs and adhesive systems to pulp exposures mechanically created in rat and nonhuman primate teeth could support the use of these materials in human pulps (Cox et al, Onoe, Akimoto et al, Kitasato et al). Additionally, in a few clinical cases in which adhesive systems were applied on acid-etched human pulps, the patients did not report any posttreatment tooth sensitivity (de Souza Costa et al 2001, de Souza Costa et al 2003c). Radiographs of these teeth taken a couple months after the procedures did not evidence the occurrence of periapical lesions. Because some animals like rodents and monkeys have a metabolism and tissue healing potential greater than that of human beings, the results of studies performed in such animal models cannot be extrapolated directly for clinical situations in humans (de Souza Costa et al 2001, de Souza Costa et al 2003c, de Souza Costa et al 2014). Also, lack of posttreatment tooth sensitivity does not ensure that specific clinical procedures are safe (de Souza Costa et al 2003c, de Souza Costa et al 2014, de Souza Costa et al 2006, de Souza Costa et al 2011, de Souza Costa et al 2002a, de Souza Costa et al 2002b, Ribeiro et al, Soares et al 2016b). When RMGICs and adhesive systems were labeled as adequate options for pulp therapy, the scientific evidence available, particularly in human teeth, was not sufficient to ensure clinical safety.

About two decades ago, Nascimento et al assessed the response of human pulps following application of Vitrebond directly on pulp exposures created in human premolars. The cavities were restored using the SE adhesive system Clearfil Liner Bond 2V (Kuraray) and composite resin. The teeth were extracted after 5, 30, or 120 to 300 days and prepared according to routine histologic techniques. At 5 days, Vitrebond elicited a moderate to intense pulpal inflammation and caused a deep necrotic zone. With time, the high amount of Vitrebond components displaced into the pulp triggered a persistent inflammatory reaction that did not allow tissue repair, defined by complete dentin bridge formation, even after long-term follow-up periods (300 days). The authors concluded that Vitrebond cannot be recommended for direct application to pulpal wounds. In some specific clinical situations in which accidental pulp exposures occurred during cavity preparation, Vitrebond was also used as a capping agent (de Souza Costa et al 2003a, de Souza Costa et al 2006, de Souza Costa et al 2011). The histopathologic analysis of these teeth showed severe pulp responses similar to those described previously (Nascimento et al).

In another study performed in human teeth, mechanically exposed pulps were capped with different components from a three-step ER adhesive system (Accorinte et al). The pulpal wounds of these teeth were conditioned with an acidic agent (step one) followed by application of the primer (step two) and bonding agent (step three). In another group of teeth, only the primer or bonding agent was applied to the pulp exposures. The cavity walls were carefully restored with composite resin. The authors showed that these treatments produced pulpal damage and did not allow complete pulpal healing (Accorinte et al). In this way, the primer and bonding agent with or without etching were not considered biocompatible. Based on these results, it was suggested that the step of acid etching does not intensify the tissue damage caused by the complete adhesive treatment.

In a pilot study performed by the de Souza Costa research group (unpublished data), pulp exposures performed in human premolars were conditioned with 32% phosphoric acid for 10 seconds. In another group of teeth, the pulp exposures were directly capped with an adhesive system whereby the primer and bonding agents were present in one bottle. All teeth were extracted 30 minutes after the procedures. When the pulp tissue was only acid etched, a thin, superficial cauterization layer was observed. However, in those pulps capped with the adhesive system, displacement of resin components occurred further down into the connective tissue. A number of dilated and congested blood vessels, plasma extravasation, and inflammation were observed. These histologic findings demonstrated that rather than the acidic agent, the monomer-rich adhesive system was the primary culprit responsible for the intense pulpal damage.

Numerous studies have evaluated the response of human pulps capped with different adhesive systems (de Souza Costa research group, unpublished data). These studies showed that inadequate pulp therapy causes a chronic inflammatory reaction mediated by mononuclear and giant cells that appear to be engulfing globules or fragments of resin displaced into the pulp tissue (Fig 10-24). It is interesting to report that in all these clinical/histopathologic investigations, the patients did not claim any postoperative tooth sensitivity. The persistent pulpal damage caused by resin components in the pulp does not allow for tissue healing, negatively interfering

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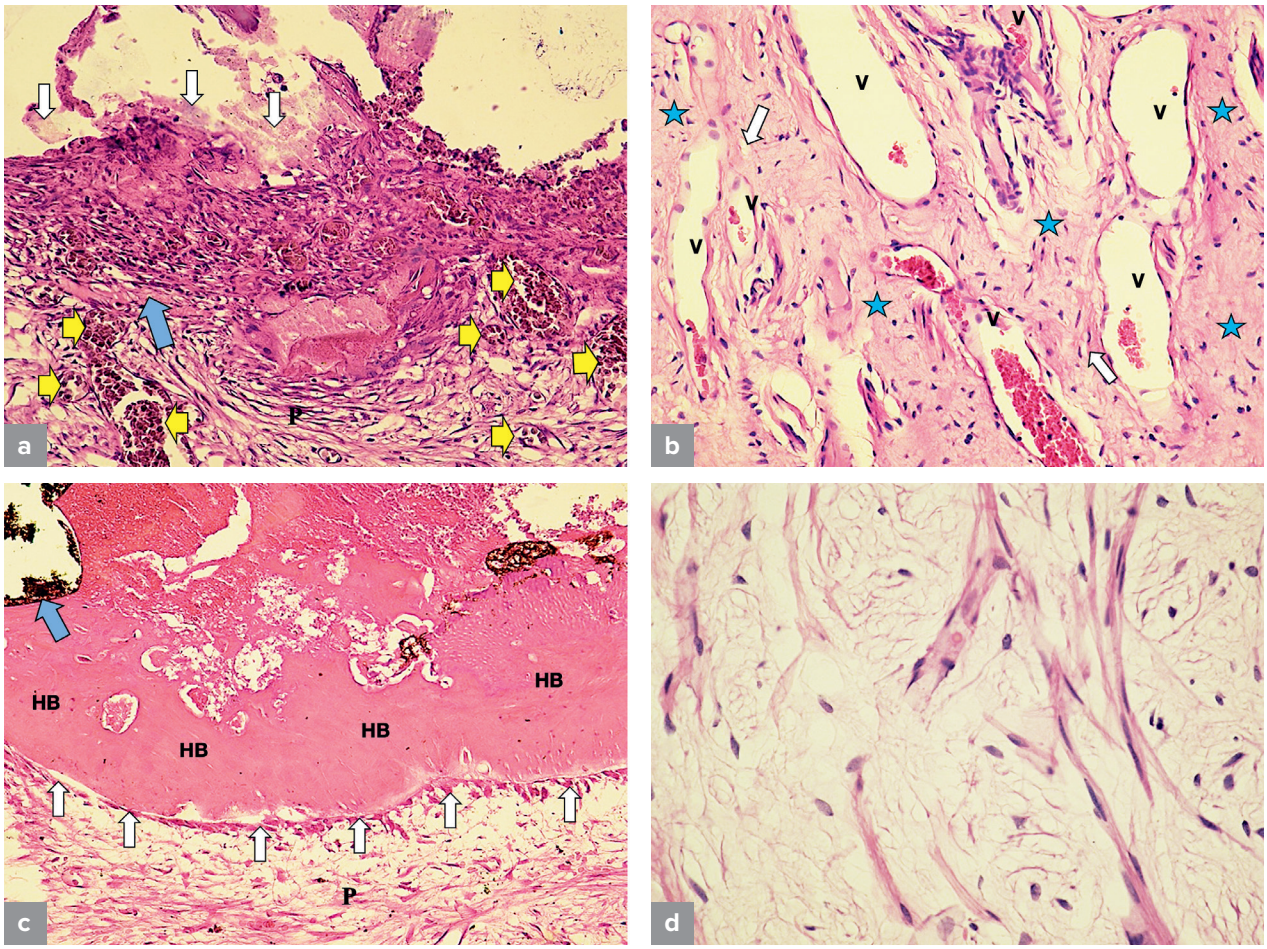


▲ **FIG 10-24** *(a)* The pulp tissue (P) of a human tooth was mechanically exposed and capped with an adhesive system. The intense chronic inflammatory reaction is characterized by several dilated and congested blood vessels *(yellow arrows)* among a number of mononuclear and a few giant cells. Note the resin-based material *(white arrows)* on the pulpal wound adhesive. Large fragments of the capping agent displaced into the pulp can also be observed *(blue arrow)* (h&e staining; original magnification ×125). *(b)* Radicular pulp tissue of a tooth in which an adhesive system was used as a capping agent. A number of dilated and congested blood vessels (V) associated with intense plasma extravasation are observed. The extracellular matrix exhibits hyalin alteration *(stars)*, and pulp cells show hydropic degeneration *(white arrows)* (h&e staining; original magnification ×180). *(c)* The pulp tissue of a human tooth was mechanically exposed and capped with calcium hydroxide powder. A continuous layer of differentiated odontoblast-like cells *(white arrows)* is underlying the complete hard tissue barrier (HB) formed below the pulp capping agent *(blue arrow)*. Note that the subjacent connective tissue presents no signs of local inflammation (h&e staining; original magnification ×125). This specific mechanism of tissue healing, which has been observed a few months after the application of calcium-based dental materials to pulp exposures, does not take place when resin-based materials such as RMGICs and adhesive systems are used as capping agents. *(d)* Radicular pulp tissue of a tooth in which the pulpal wound was capped with calcium hydroxide powder. Note that this connective tissue shows normal histologic characteristics, reflected by a number of pulp cells among a collagen-rich extracellular matrix (h&e staining; original magnification ×250).

with the complete formation of a hard tissue barrier at the pulp exposure site (Hebling et al, Pereira et al, de Souza Costa et al 2001, Gwinnett and Tay). Therefore, resin-based materials are not recommended as direct pulp capping agents.

## Response of Dental Pulp to Calcium Hydroxide and MTA

Due to its biologic features, including the capacity to induce production of a barrier of mineralized tissue, its antibacterial activity, and its alkaline pH, calcium hydroxide


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has been the most used material for vital pulp treatment (Queiroz et al 2005, Alqahtani et al). Calcium hydroxide is commercially available in powder or paste form or incorporated into chemically curing or light-curing cements. It should be emphasized that the vehicle employed to prepare the paste or cement can interfere with the ionic dissociation (Silva et al 1997), tissue response (Nelson-Filho et al), and the capacity to cause the deposition of mineralized tissue (Leonardo et al). Consequently, the biologic effects of the various calcium hydroxide–based formulations are quite diverse.

The frequency of healing of exposed dental pulps that were treated by partial pulpotomy and calcium hydroxide dressing was 96% in young patients with traumatic exposures in both open and closed apices (Cvek 1978). In a long-term follow-up study, Ricucci et al (2023) reported a very high success rate (>89%) for direct pulp capping of teeth with reversible pulpitis using calcium hydroxide, particularly in the first 10 years after treatment. The quality of the coronal restoration was the most influential element in predicting the outcomes.

Mineral trioxide aggregate (MTA) is a dental material that is particularly effective in conservative vital pulp therapy, with high success rates (90% to 100%) in clinical, radiographic, and histopathologic studies (Oliveira et al, Paula et al 2018, Paula et al 2019). It is well tolerated by the pulp tissue, possesses antibacterial capabilities, and can stimulate the creation of mineralized tissue. Similar to calcium hydroxide, MTA can solubilize growth factors and signaling molecules stored in dentin, thereby recruiting dental pulp stem cells to the healing site (Bakland and Andreasen). Biodentine (Septodont) is a tricalcium silicate substance with similar physical and chemical qualities to MTA, and it is indicated for the same type of treatment, including pulpotomy and direct pulp capping. Randomized clinical trials, systematic reviews, and meta-analyses comparing MTA and Biodentine have not demonstrated differences in clinical and radiographic success between these two materials (Stringhini Junior et al, Bossù et al, Hidalgo et al).

Calcium hydroxide, MTA, and Biodentine exert their effects via local alkalinization and calcium ion release. Within physiologic limits, an increase in pH enhances the mineralization ability of dental pulp cells and the synthesis of mineralizing proteins by odontoblasts (Sangwan et al). In addition, the alkaline pH neutralizes the acidic environment caused by the inflammatory response, favoring the repair process. The calcium ion has a chemotactic effect on cells with the capacity to produce dentin and is regarded as a significant regulator of multiple cellular processes, including proliferation, differentiation, and mineralization (Zayzafoon, Paula-Silva et al 2010b, Giraud et al, Ali and Raslan). By directly stimulating dental pulp cells and solubilizing the extracellular matrix of dentin, these materials also increase the formation of tertiary dentin (Sangwan et al).

Studies comparing the viability of dental pulp cells in contact with Biodentine and MTA have demonstrated similarity between them (Luo et al, Daltoé et al). In addition, these materials induce the formation of mineralized tissue in procedures such as pulpotomies and pulp capping because they stimulate alkaline phosphatase, osteopontin, and RUNX2 gene expression (Chang et al 2014, Daltoé et al, Almeida-Júnior et al). Specifically regarding modulation of the inflammatory response, Biodentine promoted a more intense reaction compared to MTA, showing higher expression of TNF-α, IFN-γ, and interleukin 10 (IL-10) 7 days after implantation in subcutaneous connective tissue (Silva et al 2022). However, after 15, 21, and 63 days, there was a significant reduction in the number of inflammatory cells and the immunoexpression of IL-6 for both materials (Silva et al 2022).

## Pulpal Irritants and Their Clinical Effects

A critical component for pulpal inflammation is a disruption in the transudation of dentinal fluid in the tubules, which can come from caries, crack propagation, the effects of aging, and most certainly from restorative procedures. As such, the risk factors for eliciting pulpitis should be understood and taken into account during restorative procedures, pulpal status should be monitored during treatments, and any inflammation of the pulp should be reduced as much as possible during and after restorative procedures. In addition, it is important to clearly distinguish between reversible and irreversible pulpitis so that, if necessary, prompt endodontic treatment can be performed to provide the most favorable prognosis for the patient.

Restorative dentists attempt to improve the health of the tooth and the pulp by performing treatment to remove caries and place restorations; however, each time the tooth is worked on, pulpal stress occurs (Abou-Rass). The concept of a stressed pulp must be considered before any extensive restorative dentistry is initiated, because repeated insults will lead to the deterioration of the pulp. Clinically, there is not a set threshold for when pulpal disease will occur; it varies per patient, per tooth, per dental technique applied, and per procedure completed.

Younger patients have larger dentinal tubules and more coronally positioned pulp horns (Maeda). Reeves and Stanley demonstrated that pulpal pathosis increases when the distance from the bacterial penetration to the pulp is 1 mm or less. Therefore, even small to moderate-sized caries lesions and restorations can result in increased

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pulpal inflammation for young teeth, and no matter the patient age, the depth of caries and bacterial penetration impacts the status of the pulp.

While the size of the pulp chamber and depth of bacterial penetration are patient factors, there are procedural measures and techniques that restorative dentists can control to reduce any further pulpal inflammation. The use of an effective water coolant during high-speed preparation is advised to avoid increasing pulpal temperature (Zach and Cohen 1962). It should be confirmed that this water spray is reaching the preparation site with sufficient coverage to continually wash the field (Langeland and Langeland). When inspecting the preparation, prolonged air drying should be avoided to prevent desiccation and displacement of the odontoblastic layer into the dentinal tubules, which will lead to pulpal inflammation (Cotton). Furthermore, the use of an acid etch during the placement of restorations will remove the dentinal smear layer created by the bur and allow bacterial penetration until sealed (Bergenholtz 1991). By preparing the dentin, the dentinal tubules are opened, which provides a more direct route of inflammation to the pulp. Efforts should be taken to minimize damage to the tubules, and restorations should close off the tubules to further bacterial penetration.

One of the most important factors that a restorative dentist controls in reducing pulpal inflammation and postoperative sensitivity is ensuring a tight marginal seal for restorations. Barnett suggested that most pulpal inflammatory lesions can be attributed to the leakage of bacteria and bacterial substances from the oral environment along gaps in the material-tooth interface. Composite resin undergoes volumetric shrinkage during polymerization, which can lead to gap formation if these stresses are not properly managed (Davidson et al). This gap from polymerization shrinkage precludes a completely tight seal against bacterial infiltration and is likely a contributing factor to the higher incidence of teeth requiring endodontic treatment when restored with composite. Kwang et al found that teeth restored with composite resin were 1.91 times more likely than those restored with amalgam and 5.69 times more likely than those with crowns to require subsequent endodontic intervention, and the critical time-lapse was 9 months from the time of restoration to the time of requiring endodontic therapy.

Many techniques can be employed to prevent bacterial contamination of a prepared tooth and minimize the microgap formed from the contraction stress of composite resins. Simply stated, if the stress of polymerization exceeds the strength of the developing bond, a microgap formation will result (Unterbrink and Liebenberg). The presence of a microgap will have consequences for the tooth and patient, including postoperative sensitivity, marginal leakage, and recurrent caries (Turkistani et al). In vital teeth, the gaps will fill with pulpal fluid via transudation, which alters the hydrodynamic fluid flow in the tubules, thereby eliciting sensitivity and pulpal inflammation (Brännström).

Rubber dam isolation is advised when preparing and restoring teeth to eliminate contamination in the adhesive bond necessary for composite restorations. Nair et al evaluated various adhesive systems and found that 64.6% were prone to having a deleterious impact due to salivary contamination. Saliva moisture can be trapped in the adhesive resin and compromise the overall bonding and strength of the adhesive. Another technique to improve bond strength is to utilize air abrasion. Air abrasion will condition the dentin substrate and compact the smear layer, enabling an improved adhesive bond (Mujdeci and Gorkay, Van Meerbeek et al 2003a). Additionally, immediate dentinal sealing with a compatible material should be considered to eliminate exposure of dentinal tubules, reduce pulpal inflammation, and thereby minimize patient postoperative sensitivity (Magne et al 2005, Magne et al 2007). Cox and Suzuki described postoperative hypersensitivity as a result of the breakdown of the smear layer, so sealing the dentin interface will therefore reduce this discomfort. This technique can be especially helpful following crown preparation before the temporization phase and will offer some protection to the prepared tooth structure against any thermal effects that could occur during the fabrication of the temporary crown (Tjan et al). Completion of this step early on protects the dentin-pulp complex from the ingress of bacteria (Magne et al 2005, Magne et al 2007). Any leakage around the temporary crown is of less concern if the dentinal tubules have been sealed. Additionally, salivary contamination is also reduced when removing the temporary crown and seating the permanent crown. It is an easy step to incorporate into the restorative protocol with multiple advantages for the patient and the dental pulp (Magne et al 2005, Magne et al 2007). It is also important to note that dentists need to be aware of the potential toxic effects of bonding agents to the pulp cells. As previously mentioned, damage to the pulp can be observed when the remaining dentin thickness is less than 0.3 to 0.5 mm.

Any steps an operator can take to reduce pulpal inflammation are helpful, but pulp necrosis may still occur. Felton et al reported higher incidences of pulpal necrosis in teeth with full-coverage restorations (13.3%) compared to partial veneer restorations (5.1%). In a dye-leakage study, Goldman et al showed that all margin preparation types (chamfer, shoulder, shoulder + bevel) had leakage. Leakage can be an important causative factor in pulpal inflammation and necrosis. Teeth utilized as bridge abutments were shown to have a higher incidence of pulp


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necrosis at 32.5% compared to single-unit porcelain-fused-to-metal crowns at 15.6% (Cheung et al). Typically, a tooth that requires a crown has already had extensive restorative treatment previously and has a large caries lesion or crack(s) necessitating full-coverage restoration. Kontakiotis et al's prospective study on the incidence of asymptomatic pulp necrosis following crown preparation found that *(1)* in a time frame of 6 to 10 weeks, 9% of teeth were diagnosed with asymptomatic pulp necrosis; *(2)* teeth that were intact preoperatively had a 5% incidence of necrosis, while those with preoperative restorations and cracks had a 13% incidence of necrosis; and *(3)* there was an eightfold increase in incidence of pulp necrosis in teeth with preoperative caries, fillings, or crowns. The process of crown preparation is also relatively invasive to the tooth, and the pulp in these teeth has already been repeatedly stressed and is therefore at higher risk of pulpal deterioration (Abou-Rass).

Another challenge in managing pulpal inflammation is distinguishing what is within normal limits of postoperative sensitivity and reversible pulpitis for a patient and then identifying when there has been a transition to irreversible pulpitis requiring endodontic treatment. It is smart practice to obtain a preoperative pulpal diagnosis and periapical radiograph before initiating any restorative treatment. If there is already evidence that endodontic treatment is necessary, it can be completed before delivery of the permanent restoration. If the preoperative diagnostic step is overlooked and the patient comes back following treatment with pain, the perception is likely that it is due to the recent treatment and that the dentist has "caused" the need for a root canal. Obtaining this preoperative data and diagnosis establishes a baseline and allows for a conversation with the patient about their existing pulpal status and potential risks for pulpal inflammation with the proposed restorative treatment. Percussion and cold testing are relatively quick and easy to complete for this baseline diagnosis, along with a periapical radiograph to evaluate any preexisting apical pathosis. During treatment phases, particularly in any temporization phase, monitoring of the patient with repeated sensibility testing by the restorative dentist can ensure the maintenance of normal pulp or reversible pulpitis. Kwang et al found 9 months to be the critical time-lapse from restoration to requiring endodontic treatment. Therefore, it is suggested to complete sensibility testing at future patient encounters—perhaps at their next periodic evaluation or hygiene appointment—to thoroughly monitor the pulpal diagnosis. If at any time a patient expresses an increased and lingering sensitivity to temperatures, spontaneous throbbing, or increased bite pressure sensitivity, their pulpal status has likely degraded to an irreversible or necrotic state and endodontic treatment is advised.

A review of the literature shows that some incidence of pulpal inflammation and necrosis is inevitable even with best practices utilized in restorative procedures. It is therefore important to communicate this risk to the patient, minimize irritation during treatments, and follow up with sensibility testing and endodontic referral if necessary.

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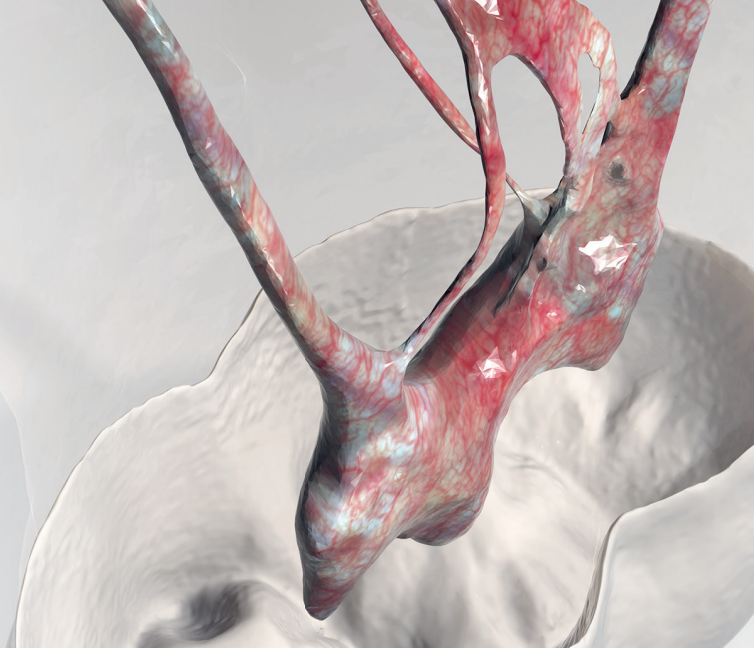
# 11

# STEM CELLS AND REGENERATION OF THE DENTIN-PULP COMPLEX

Peter E. Murray
Franklin García-Godoy



The stem cell development of teeth and the regeneration of dentin-pulp injuries affect the daily quality of life of nearly every human on this planet through their ability to eat, speak, smile, and—when regeneration fails—experience toothache (Garcia-Godoy et al). Tooth development, regeneration, and repair rely on the ability of functional dental stem cells to differentiate into all the different cell types found in the dentin-pulp complex (Morsczeck and Reichert). The tooth germ, or tooth bud, houses the most versatile and significant dental stem cells, as these stem cells can create whole teeth in all mammals (Matalová et al). During the early stages of tooth development, the dental papilla stem cells produce odontoblasts that synthesize dentin (through dentinogenesis) as well as fibroblasts, subodontoblasts, nerves, and blood vessels, which comprises most of the native cells within the dentin-pulp complex. Dental follicle mesenchymal progenitor stem cells surround the tooth germ to create the cementum, periodontal ligament, and alveolar bone around the tooth (Zhou et al). Ameloblasts arise from stem cells within the enamel organ, and these cells synthesize enamel through amelogenesis.

Mature teeth have an apical foramen that is nearly closed, and almost all of the tooth germ stem cells will have died once teeth are mature. The scarcity of stem cells in fully mature human teeth limits the regenerative responses of enamel, cementum, and the dentin-pulp complex. Mature human teeth therefore have weak regenerative responses to injury compared to continually growing rodent teeth. However, during the late stages of development, tooth germs create several distinct populations of dental stem cells (Box 11-1), some of which may persist during the lifetime of the human tooth by residing around blood vessels in the dentin-pulp complex, similar to pericytes (Alliot-Licht et al). The hematopoietic stem cells (HSCs) in bone marrow produce the majority of the mineral-forming pericytes and perivascular stem cells (Crisan et al). These HSCs can be sequestered in the dentin-pulp complex throughout life, where they remain quiescent until activated by signals of an injury (see Box 11-1). The HSCs may also form pulp calcifications, or pulp stones, and contribute to increased extracellular matrix deposition and fibrosis of the dental pulp tissue during aging.

The most important types of dental stem cells are periodontal stem cells (PSCs), endothelial stem cells, periapical stem cells, nerve stem cells, stem cells from human exfoliated primary teeth, and dental pulp stem cells (DPSCs). DPSCs can respond to dentin-pulp injury

**BOX 11-1 Types of stem cells and their origins within the dentin-pulp complex**

**Types of stem cells**

- Dental pulp stem cells
- Odontoblastoid cells
- Odontoblast-like cells
- Stem cells from human primary teeth
- Stem cells from apical papilla
- Stem cells from nerves and endothelial and periapical tissues

**Major origins of dentin-pulp stem cells**

1. Undifferentiated stem cells created by the tooth bud stem cells during tooth development and residing in the pulp throughout life (around blood vessels, similar to pericytes)
2. Hematopoietic stem cells from bone marrow delivered by the blood during revascularization

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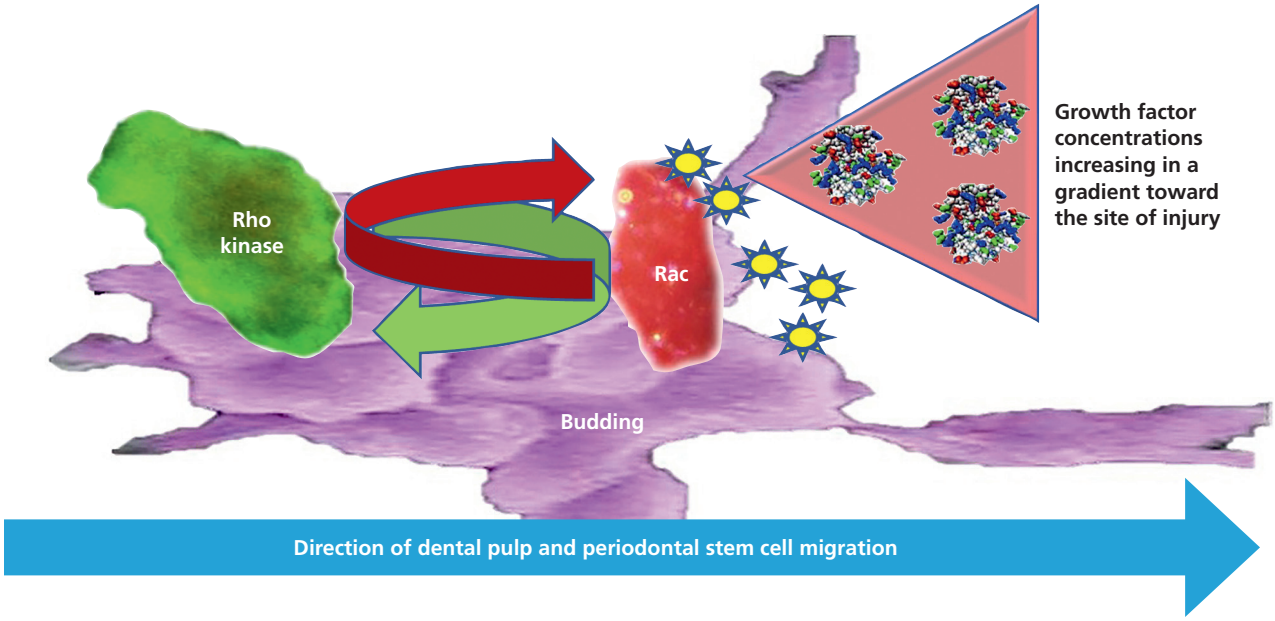
Growth factor concentrations increasing in a gradient toward the site of injury
Rho kinase
Rac
Budding
Direction of dental pulp and periodontal stem cell migration

▲ **FIG 11-1** Theoretical composite image of a dental stem cell migration response. DPSCs and periodontal stem cells have the physiologic capability to change their shape using actin stress fibers; for example, they can create pseudopodia (arms) and ruffle their external membranes to migrate through tissues in response to increasing concentrations of growth factors detected using forward-activated membrane receptors *(yellow)*. These stem cells accelerate forward when the balance of the membrane receptors favor Rac expression *(red)* in response to growth factors. They can also stop and attach to dentin or a tissue with a damaged extracellular matrix when the balance of the membrane receptors trigger Rho kinase expression *(green)*. In addition, these stem cells can divide by budding off new progeny cells after completing a cell cycle, doubling their numbers every 2 days in cell culture.

by directed migration toward homing signals (Smith et al 2012). DPSC migration is highly regulated in response to extracellular matrix proteins, particularly laminin, and chemotactants, particularly spingosine-1-phosphate and transforming growth factor β1 (TGF-β1), which likely mediate stem cell regeneration responses to dentin-pulp injury (Howard et al).

Stem cells are special because of their astounding genetically programmed capabilities to dynamically alter their cell membrane shape, called *treadmilling*, and to change their appearance or phenotype from spheres to stars to elongated torpedo-like shapes with pseudopodia, filopodia, and lamellipodia, similar to functioning limbs and fingers, to be used for adhesion and crawling into tissues (Kroon et al). Stem cells prefer to reside in bone marrow and perivascular niches because they can survive on a ready supply of nutrients and oxygen. There they can exist in a quiescent state, monitoring for injury signals, and once activated, begin continually dividing to produce progeny stem cells. Stem cells can leave their niche and be transported by rolling through blood vessels to reach any tissue within the entire human body. Furthermore, they can transmigrate through the walls of blood vessels by attaching to and squeezing themselves through endothelial cells and other cell types to reach the site of injury and begin regeneration (Hu et al). Stem cells detect, identify, communicate, share information, and coordinate their activities and functions with adjacent cells through cross-talk (Wang et al 2020). They can also detect local gradients of specific growth factors because their membrane receptors can activate molecular pathways (Park et al). The activated molecular pathways can induce stem cells to proliferate into millions of progeny stem cells or to produce proteins for dentinogenesis, osteogenesis, or soft tissue regeneration. These astoundingly complex activities are believed to be similar for DPSCs and PSCs (Murray 2012), although each stem cell type has important genetic differences in function (Hoang et al). A theoretical representation of a dental stem cell migrating toward a gradient of growth factors to reach a site of dental injury is shown in Fig 11-1.

Mesenchymal stem cells from bone marrow have migration (or homing) responses to reposition and transport themselves to any specific location within the body, and within the dentin-pulp tissue, to begin the regeneration of the injured tissue (Ahmed et al). Mesenchymal stem cells are delivered to root canals by the flow of blood during revascularization (Lovelace et al).


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The natural ability of stem cells to regenerate an injured dentin-pulp complex can be overwhelmed by the toxicity of a bacterial infection, necrosis, caries, and extensive trauma. Consequently, root canal disinfection, debridement, and cleaning is essential to create a nontoxic and biocompatible environment that will allow stem cells to regenerate a new replacement dentin-pulp complex to revitalize immature teeth (Murray et al 2007, Lovelace et al).

Stem cells from exfoliated primary teeth can be collected at the dental office, shipped to a tooth bank, stored, harvested when needed, and bioengineered to create replacement tissues for regenerative dental and medical treatments (Zeitlin). The outlook for the improved effectiveness of regenerative dentistry and regenerative medicine is exciting, because 89% of dentists are willing to save teeth and dental tissues for the banking of each patient's own stem cells (Epelman et al). Stem cell therapies have the potential to alleviate the future burdens of many disabilities. Although the difficulties associated with introducing new stem cell therapies are substantial, the potential benefits to patients and the profession are equally promising—a cure for caries and other oral disease, a cure for oral cancer, the correction of congenital defects, and the regeneration of teeth and tissues to restore oral function (Murray and Garcia-Godoy 2004). Recent advancements in stem cell technology have opened a door for patients suffering from diseases and disorders that have yet to be effectively treated (Hoang et al).

Dentists overwhelmingly prefer regenerative endodontics to save permanent immature natural teeth, and patients are increasingly requesting regenerative dentistry to replace missing dental tissues. Most dentists are supportive of using more regenerative procedures in their dental practice, and they are willing to undergo extra training and buy new technology to provide new stem cell regenerative procedures (Manguno et al).

In fact, dentists who regenerate tissues for their patients are employing stem cell therapies whether they realize it or not. Furthermore, almost all dentists believe that regenerative stem cell therapies will become more widely used in future dental practice (Epelman et al, Manguno et al). Stem cell regenerative therapies are rapidly evolving and are often featured in the news media, which is why patients are increasingly likely to judge the skills of their dentist according to their ability to successfully provide them with regenerative dentistry to replace missing tissues.

## Regenerative Dentistry

The development of biomimetic and nanorestorative biomaterials, in vitro cell culture technology, tissue-engineered scaffolds and membranes, molecular biology, and the human genome project have provided the basis for the introduction of new technologies into dentistry (Garcia-Godoy and Murray 2010). Regenerative dentistry seeks to regenerate replacement teeth, bone, and other dental tissues (periodontal, gingival, mucosal, etc) via dental revascularization by connecting the patient's vascular blood supply to the stem cells (Baru et al), in conjunction with tissue scaffolds, growth factors, and genetic programming (Yan et al). DPSC clinical transplantation therapies have a more than 80% success rate for bone regeneration, treatment of periodontitis, and dental pulp regeneration, and no adverse events have been reported (Yamada et al). However, not all dentists and researchers agree that regenerative dentistry will be clinically effective for regenerating whole replacement teeth and dental tissues (Amrollahi et al).

Successful regenerative dentistry requires an aseptic, nontoxic, non-necrotic, and non–severely inflamed environment that is suitable for new tissue regeneration (Murray et al 2007, Ring et al). This often requires the removal of infected, necrotic, irreversibly inflamed, and/or caries-diseased tissues. New tissue regeneration requires a source of progenitor stem cells that may need to be harvested, isolated, and/or stimulated with growth factors and/or genetic engineering to proliferate the regeneration of the millions of cells needed to create a functional replacement dentin-pulp tissue. True stem cells are extremely rare, and HSCs comprise only 1 out of every 10,000 bone marrow mononucleated cells (Kumaravelu et al). It is estimated that the average human body contains a total of $3.0 \times 10^{13}$ cells, and it contains an equal number of bacteria (Sender et al). Thus, fewer than one billion stem cells are available for the whole human body for all its healing, repair, and regeneration requirements, and most of these stem cells may be unavailable because they remain fixed in the bone marrow or other tissue niches, are immature or expired, or are simply inappropriate types of stem cells for this type of regeneration. Tissue regeneration is therefore undoubtedly restricted by the scarcity of suitable stem cells. However, researchers are generally seeking to augment the numbers of stem cells suitable for regeneration and exponentially increase regeneration by stem cell banking (Khaseb et al). Most stem cell therapies are injected or surgically implanted at a local site of injury and require revascularization to stimulate healing and regeneration.

Dentin-pulp progenitor stem cells are genetically programmed to produce specific types of functional odontoblast-like, osteoblast-like, or mucosal-like cells. If progenitor stem cells are not already genetically programmed to create an appropriate dentin-pulp tissue, they can be genetically engineered. DPSCs and PSCs have a special ability to migrate to specific organizational

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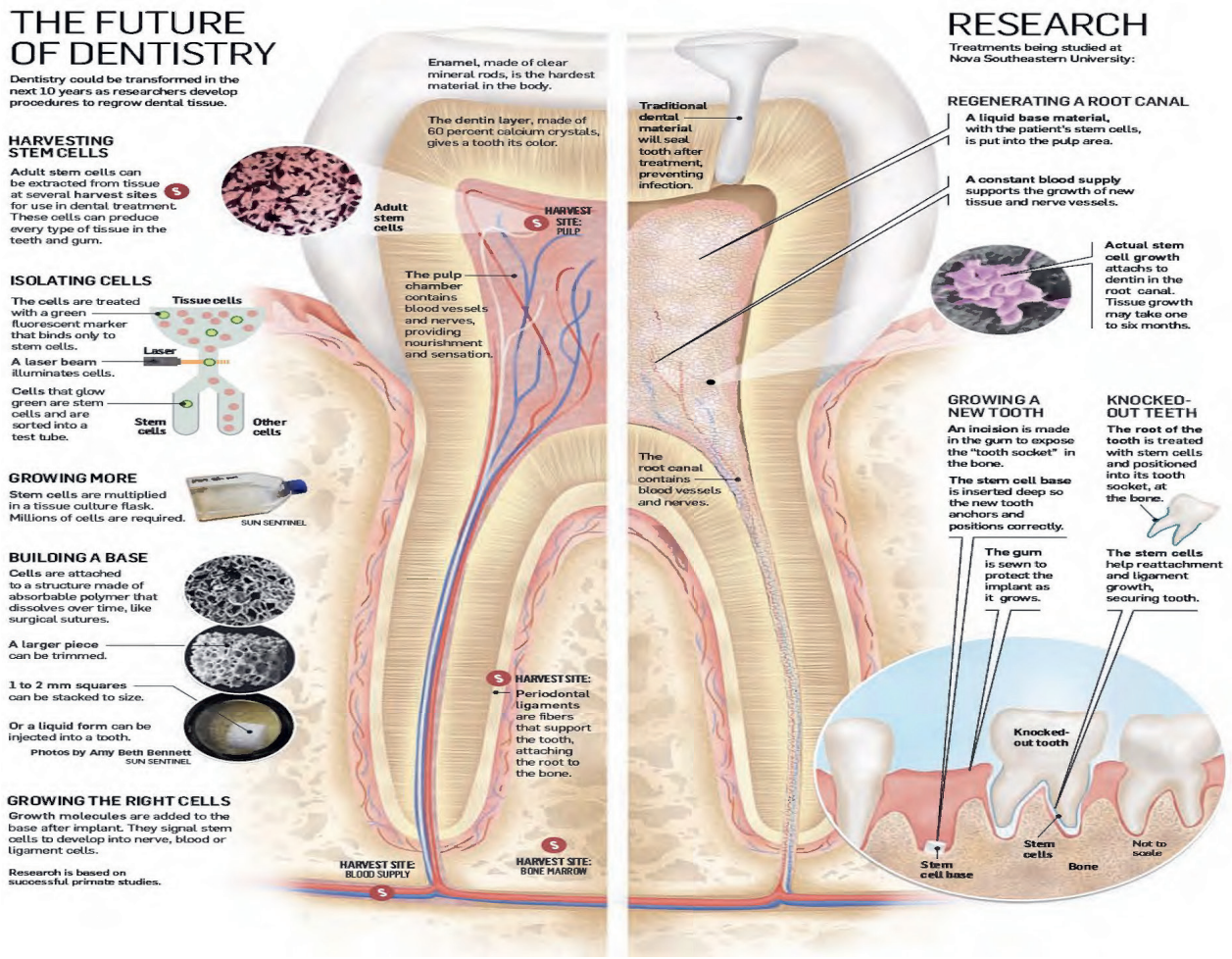
THE FUTURE OF DENTISTRY
Dentistry could be transformed in the next 10 years as researchers develop procedures to regrow dental tissue.
HARVESTING STEM CELLS
Adult stem cells can be extracted from tissue at several harvest sites for use in dental treatment. These cells can produce every type of tissue in the teeth and gum.
ISOLATING CELLS
The cells are treated with a green fluorescent marker that binds only to stem cells.
Tissue cells
Laser
A laser beam illuminates cells.
Cells that glow green are stem cells and are sorted into a test tube.
Stem cells
Other cells
GROWING MORE
Stem cells are multiplied in a tissue culture flask. Millions of cells are required.
SUN SENTINEL
BUILDING A BASE
Cells are attached to a structure made of absorbable polymer that dissolves over time, like surgical sutures.
A larger piece can be trimmed.
1 to 2 mm squares can be stacked to size.
Or a liquid form can be injected into a tooth.
Photos by Amy Beth Bennett SUN SENTINEL
GROWING THE RIGHT CELLS
Growth molecules are added to the base after implant. They signal stem cells to develop into nerve, blood or ligament cells.
Research is based on successful primate studies.
Enamel, made of clear mineral rods, is the hardest material in the body.
The dentin layer, made of 60 percent calcium crystals, gives a tooth its color.
Adult stem cells
HARVEST SITE: PULP
The pulp chamber contains blood vessels and nerves, providing nourishment and sensation.
HARVEST SITE: Periodontal ligaments are fibers that support the tooth, attaching the root to the bone.
HARVEST SITE: BLOOD SUPPLY
HARVEST SITE: BONE MARROW
RESEARCH
Treatments being studied at Nova Southeastern University:
REGENERATING A ROOT CANAL
A liquid base material, with the patient's stem cells, is put into the pulp area.
A constant blood supply supports the growth of new tissue and nerve vessels.
Traditional dental material will seal tooth after treatment, preventing infection.
The root canal contains blood vessels and nerves.
Actual stem cell growth attachs to dentin in the root canal. Tissue growth may take one to six months.
GROWING A NEW TOOTH
An incision is made in the gum to expose the "tooth socket" in the bone.
The stem cell base is inserted deep so the new tooth anchors and positions correctly.
The gum is sewn to protect the implant as it grows.
KNOCKED-OUT TEETH
The root of the tooth is treated with stem cells and positioned into its tooth socket, at the bone.
The stem cells help reattachment and ligament growth, securing tooth.
Knocked-out tooth
Stem cell base
Stem cells
Bone
Not to scale

▲ **FIG 11-2** The future of dentistry is using stem cells to bioengineer replacement tissues for patients. Viable cells are harvested from extracted teeth or bone marrow, and then stem cells are isolated using markers by flow cytometry to remove the non–stem cells. The stem cells are proliferated into millions by growing them in an incubator, and then they are seeded onto a base of scaffolds inside a bioreactor to create 3D tissues, such as cloning a replacement tooth to replace one that was knocked out. (This image is republished with permission from the *Sun Sentinel* article titled "The Tooth Harvest.")

sites within a 3D space to create a new functional tissue (Gebhardt et al). Dentin-pulp stem cells can have various origins and have been given several names, but all share the unique ability to become functional odontoblast-like cells that produce dentin. A review of the use of dentin-pulp stem cells to clone replacement teeth and to bioengineer tissues for patients is shown in Fig 11-2.

## Regeneration of Dentin-Pulp Tissues

Dentin-pulp and dental tissues are created by PSCs, DPSCs, endothelial stem cells, periapical stem cells, nerve stem cells, and stem cells from human exfoliated primary teeth. Bone-forming pericytes originate from HSCs and may be sequestered in the dentin-pulp complex throughout life, where they remain quiescent until activated by an injury stimulus. These pericytes may form pulp calcifications and increase dentin-pulp mineralization during aging. HSCs can respond to dentin-pulp injury by directed migration toward homing signals and can also be delivered to the site of dentin-pulp injury by the blood flow from revascularization. The natural readiness of stem cells to regenerate the injured dentin-pulp complex can be overwhelmed by bacterial infection, necrosis, severe caries disease, and critical-size traumatic tissue injury.

## Regeneration of Immature Permanent Teeth

The regeneration of immature permanent teeth following trauma could be beneficial to reduce the risk of fracture and loss of millions of teeth each year (Garcia-Godoy and Murray 2012). Regenerative endodontics, revascularization, apexification, apexogenesis, and traditional root


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canal treatments have become essential treatments to save injured and diseased immature permanent teeth (Murray 2023). Regenerative endodontics can involve revascularization and/or autologous platelet-rich plasma and scaffolds to elicit host stem cell de novo tissue formation, especially root maturation and apical closure (Murray 2018). The goal is to re-establish the vitality of immature teeth for the purpose of continuing root maturation (American Association of Endodontists). Regenerative endodontics promotes dentinogenesis within the dentin-pulp complex to strengthen the fragile thin dentinal walls of immature teeth, thereby protecting the tooth from being lost due to overload stress and fracture (He et al, Trope).

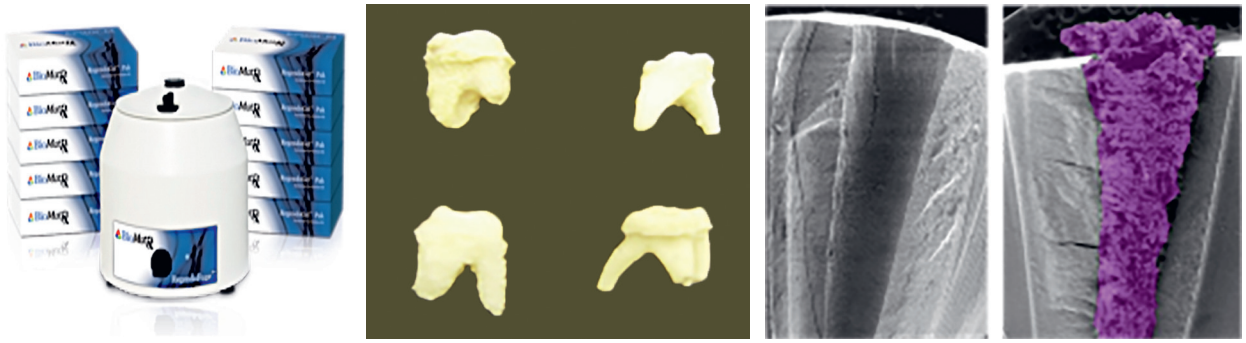
Regenerative stem cell treatments can be successful by adhering to case selection criteria, using precise delivery steps, and carefully monitoring the outcomes to identify and re-treat any failures (American Association of Endodontists). Uniquely, regenerative endodontics has the potential to continue the root development of immature permanent teeth, thereby potentially saving the teeth for the lifetime of the patient (Murray 2023). Nonetheless, conventional endodontic root canal treatment, partial pulpotomy, apexogenesis, and apexification should always be provided in situations where these treatments are more likely to benefit the patient.

## Dental Chairside Regeneration Assays

Clinicians want a relatively quick, inexpensive, and convenient method to deliver stem cell regeneration therapies to their patients, such as sheets of human DPSCs for bone replacement, via dental chairside assay (Pedroni et al). Tissue regeneration chairside assay kits have become commercially available, mostly using biodegradable scaffolds or phlebotomy-derived scaffolds. Regeneration assays require the dentist to employ three phases: *(1)* Isolate the patient's own stem cells; *(2)* seed the patient's own stem cells into a biodegradable 3D scaffold to create a tissue construct; and *(3)* surgically implant the tissue construct into the patient to regenerate new replacement tissue. These phases for using dental chairside regenerative assays are summarized in Fig 11-3.

## Stem Cell Banking and Bioengineering of Replacement Tissues

Babies usually develop primary teeth between 6 and 12 months of age, with an average eruption age of 6.6 months (Zhang et al). Primary teeth naturally begin to fall out when the child reaches the age of 4 to 7 years and are replaced by permanent teeth. However, now that it has



**Stem cell isolation and the creation of a scaffold**

Blood is collected from the patient and centrifuged to separate stem cells and platelet-rich fibrin or platelet-rich plasma.

**Tissue constructs**

Stem cells and/or scaffolds are combined to create tissue constructs.

**Surgery**

The tissue constructs are surgically implanted and revascularized into the root canals of teeth or are used to replace other missing dental tissues.

▲ **FIG 11-3** A dental chairside assay can be used to regenerate replacement tissues by obtaining stem cells and/or a scaffold matrix from the patient's blood. The blood is drawn from the patient's vein and then centrifuged to obtain platelet-rich plasma or platelet-rich fibrin. These are used as a revascularization scaffold or combined with stem cells to regenerate replacement tissues.

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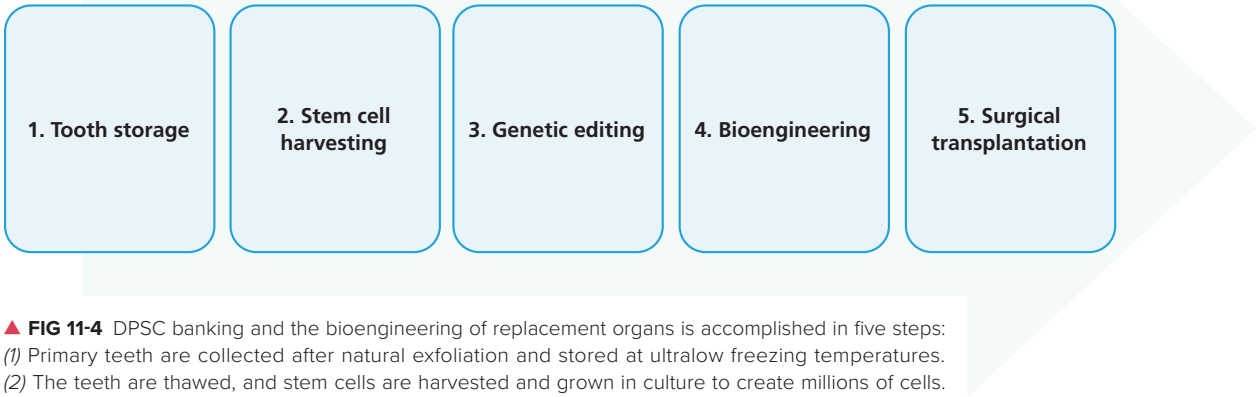


▲ **FIG 11-4** DPSC banking and the bioengineering of replacement organs is accomplished in five steps: *(1)* Primary teeth are collected after natural exfoliation and stored at ultralow freezing temperatures. *(2)* The teeth are thawed, and stem cells are harvested and grown in culture to create millions of cells. *(3)* The stem cells are genetically edited to activate genes to create specific functional progenitor stem cells. *(4)* A new organ is bioengineered inside a bioreactor by seeding a 3D scaffold with progenitor stem cells. *(5)* The new bioengineered organ is surgically transplanted into the patient with vascularization and the connecting of nerves.

been discovered that stem cells reside within the dentin-pulp complex, these primary teeth should no longer be discarded, because they contain a valuable resource of stem cells for bioengineering and regenerating replacement tissues (Zeitlin).

Clinicians, patients, and researchers may favor the harvesting of stem cells from primary teeth instead of from umbilical cords or bone marrow because these are easily accessible and do not harm the donor. Shortly after collection, the primary teeth containing the stem cells can be maintained at ultralow freezing temperatures for decades. Whenever the donor patient requires a replacement organ, the tooth bank thaws the stem cells and grows them in cell culture to create millions of stem cells for harvesting to generate a replacement tissue. The DNA within the genes of the stem cells can be edited using clustered regularly interspaced short palindromic repeats (CRISPR) technology to activate the specific gene expression for the particular type of organ required (Wang et al 2017). In the bioengineering phase, the progenitor stem cells can be seeded onto biodegradable scaffolds called *tissue constructs* to create a 3D size and shape of the target tissue to be regenerated, such as a biomimetic tooth germ or a bud model (Monteiro et al). The organ construct is provided with nutrients, energy, and oxygen inside a bioreactor for the stem cells to create a functional organ. In the transplantation phase, the bioengineered organ is surgically transplanted into the recipient patient by connecting the nerves and blood supply by revascularization. A diagram of these phases is shown in Fig 11-4.

Stem cell therapies will transform future medical practice by regenerating replacement tissues and organs. DPSCs from primary teeth stored in cryogenic banks have a similar tissue regeneration potential to umbilical cord stem cells but are much easier to collect. Researchers can use gene editing to reprogram stem cells (Yan et al), which theoretically can enable DPSCs to create any tissue or organ that a sick or injured patient could need, such as replacement skin, bone, or even a heart or liver.

The patients who would benefit most from dental stem cell regeneration therapies are those missing functional and esthetic tissues due to malformation, disease, infection, necrosis, or trauma. The projected annual demand for stem cell–based dental treatments in the United States can be estimated at over 290 million tooth restorative treatments and over 30,000 patients requiring tissue regeneration following craniofacial cancer surgery (Table 11-1). Professional dental treatments cost Americans over $60 billion per year (Garcia-Godoy and Murray 2006), and scientific advances in stem cell technologies, tissue engineering, and transplantation will provide the basis for the introduction of exciting new treatment technologies into dentistry (Garcia-Godoy and Murray 2006).


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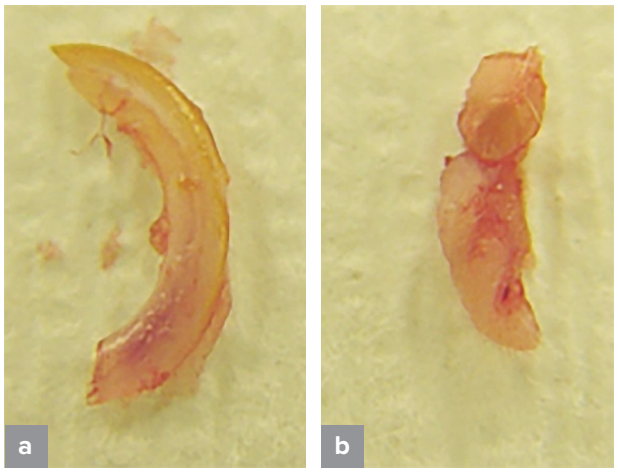
**TABLE 11-1** Potential number of stem cell regenerative dental treatments annually in the United States

| Type of treatment | Number of patients requiring treatment |
|---|---|
| Restorative dental treatment | 290 million |
| Periodontal therapy | 20 million |
| Endodontic nonsurgical root canal treatment | 16 million |
| Endodontic periapical surgery | 4 million |
| Dental implants | 2 million |
| Reconstructive treatment following dental trauma | 500,000 |
| Cleft palate and birth defect treatment | 250,000 |
| Oral cancer treatment | 34,000 |




▲ **FIG 11-5** *(a)* A natural mouse incisor with uniform size and shape. *(b)* A bioengineered mouse incisor can be misshapen due to its different origins and developmental environment.

## Cloning Teeth

Astonishingly, it is possible to clone entire teeth in mice using tooth germs (Li et al). Tooth germs surgically implanted on a biodegradable scaffold will erupt as a bioengineered tooth (Stutz et al). Cloned and natural rodent teeth share some similarity in their specific size, shape, and anatomical structure (Fig 11-5). Nonetheless, the excitement about cloning teeth is tempered by the health risk of the implanted tooth germ migrating from the site of bone implantation and developing a cloned tooth in the tongue (unpublished). The migration risks of cloning teeth and tissues can be reduced by anchoring the stem cells within a tissue construct prior to surgical implantation. The emergence of private clinics proclaiming the effectiveness of stem cell therapy as "magic cells" has raised highly publicized concerns about the efficacy and safety of stem cell therapy. The most notable adverse event of a clinical trial involved the injection of stem cells into the eyes of three patients diagnosed with macular degeneration, sadly resulting in the loss of vision for these patients (Hoang et al).

## Dentin-Pulp Repair Responses to Dentin Injuries

*Dentinogenesis* is the process of dentin secretion by odontoblasts; it continues throughout the life of a vital human tooth at an average rate of 4 microns per day (Linde and Goldberg). Human teeth do not normally grow in external dimensions after maturity. Instead, due to aging, the root canal space becomes increasingly mineralized and constricted. The number of odontoblasts and cells within the human dentin-pulp complex will reduce by half between the ages of 20 and 60 years of age (Murray et al 2002c). Primary dentin is mineralized during tooth formation in early childhood, and secondary dentin is from the continued mineralization of dentin after tooth eruption (Nudel et al).

### Reactionary dentin

Caries, wear, attrition, cavity preparation, and trauma can injure the dentin-pulp complex and trigger the release of transforming growth factor β (TGF-β) (Smith et al 1995) and other bioactive molecules (Neves and Sharpe), which will stimulate a tertiary dentin repair response (Smith et al 2012) . Tertiary reactionary dentinogenesis is the intrinsic dentin repair response of the odontoblasts within the dentin-pulp complex (Tziafas) to protect the vitality of the dentin-pulp cells. If the dentin injury is a minor caries lesion or minor cavity that does not expose the underlying dental pulp, it may stimulate the underlying odontoblasts to increase their rate of dentinogenesis, known as *reactionary dentin*. This reactionary dentin is formed on the pulpal surface of existing dentin and thickens the dentin (Neves and Sharpe), as shown in Fig 11-6.

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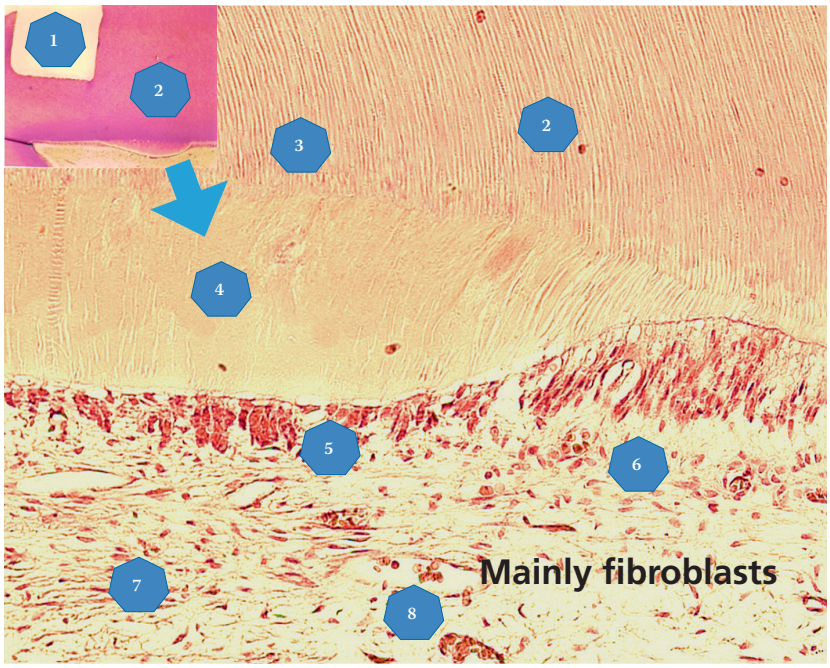


◀ **FIG 11-6** The regenerative response to tooth injury is accomplished by the increased dentinogenesis of odontoblasts to produce reactionary dentin. 1 = cavity; 2 = secondary dentin; 3 = damaged dentin releases growth factors, which signals injury for repair; 4 = reactionary dentin (tertiary dentin repair response); 5 = odontoblasts beneath the injured tubules increase their rate of dentinogenesis; 6 = subodontoblasts form a layer of supporting cells adjacent to the odontoblasts; 7 = fibroblasts are connective cells that predominate in the dental pulp; 8 = blood vessels predominantly deliver red blood cells and immune cells.

## Reparative dentin bridge

Dentin-producing odontoblasts in mature human teeth are terminally differentiated (Sun et al) and cannot multiply themselves to repopulate dead odontoblasts. After a pulp exposure destroys the odontoblasts, if there is no bacterial infection, the stem cells within the dentin-pulp complex will create replacement odontoblast-like cells. The odontoblast-like cells secrete and mineralize a reparative dentin bridge to regenerate dentin across the site of the pulp exposure. Vital pulp therapy is the direct capping of the vital exposed dental pulp with biocompatible materials such as mineral trioxide aggregate (MTA) and calcium hydroxide. Note that the volume of the dentin bridge formed with platelet-rich plasma (PRP) or platelet-rich fibrin (PRF) was greater than that formed with MTA (Shobana et al), underscoring the potential of regenerative endodontics.

Vital pulp therapy is controversial because it can have a poor long-term prognosis (Ricucci et al 2019). Traditional endodontic root canal therapy may be more beneficial for mature teeth with thick roots, and vital pulp therapy may be beneficial to strengthen immature teeth with thin roots to avoid the loss of the tooth by fracture. Bacterial microleakage, presence of a caries lesion extending into the dental pulp, and a necrotic or irreversibly inflamed pulp are all major contraindications for the success of vital pulp therapies and long-term direct pulp capping (Antonelli). Additional contraindications include any pulp exposures larger than 1 mm, excessive bleeding in the pulp, and teeth with a closed apical foramen that restricts the blood supply.

Pulp capping materials (especially those lacking biocompatibility or with marginal sealing), dentin chips, and operative debris should never be forced into an exposed dental pulp, because they disrupt the formation of the reparative dentin bridge (Murray et al 2003). MTA, Biodentine (Septodont), and similar biocompatible materials should be used for direct pulp capping or vital pulp therapy to avoid interfering with the reparative dentin bridge's ability to seal the site of dental pulp exposure (Murray and García-Godoy 2006). Pulp capping is considered successful if the tooth remains vital and a reparative dentin bridge is formed within 75 to 90 days (Pameijer and Stanley). The presence of any contraindications may prevent the reparative dentin bridge from forming, because bacteria kill the vital DPSCs and the vital dentin-pulp tissues may be replaced by necrosis (dead cells), as shown in Fig 11-7.

The tertiary dentin responses of reactionary dentin and reparative dentin bridge formation are different from one another. Whereas reactionary dentin is created by the increased rate of dentinogenesis from surviving odontoblasts (with no DPSC involvement), the reparative dentin bridge is formed when DPSCs create replacement odontoblast-like cells following major pulp exposure that destroys the existing odontoblasts (Fig 11-8).

The type of tertiary dentin reparative response is determined by the severity of the dentin-pulp injury, as more


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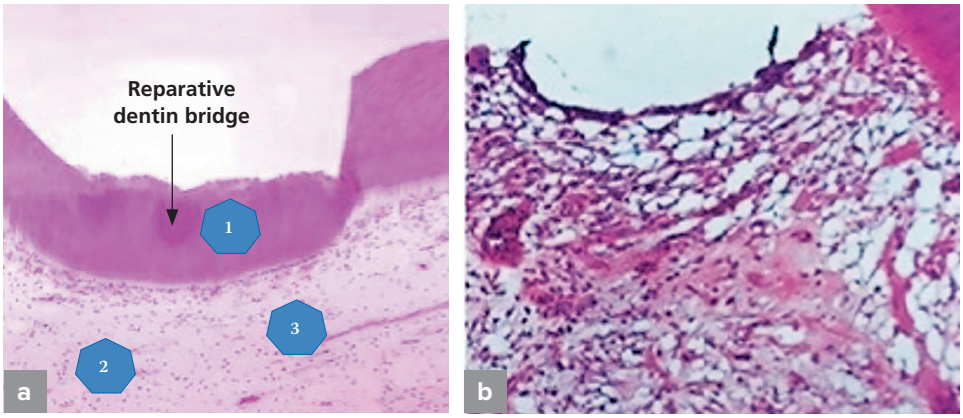
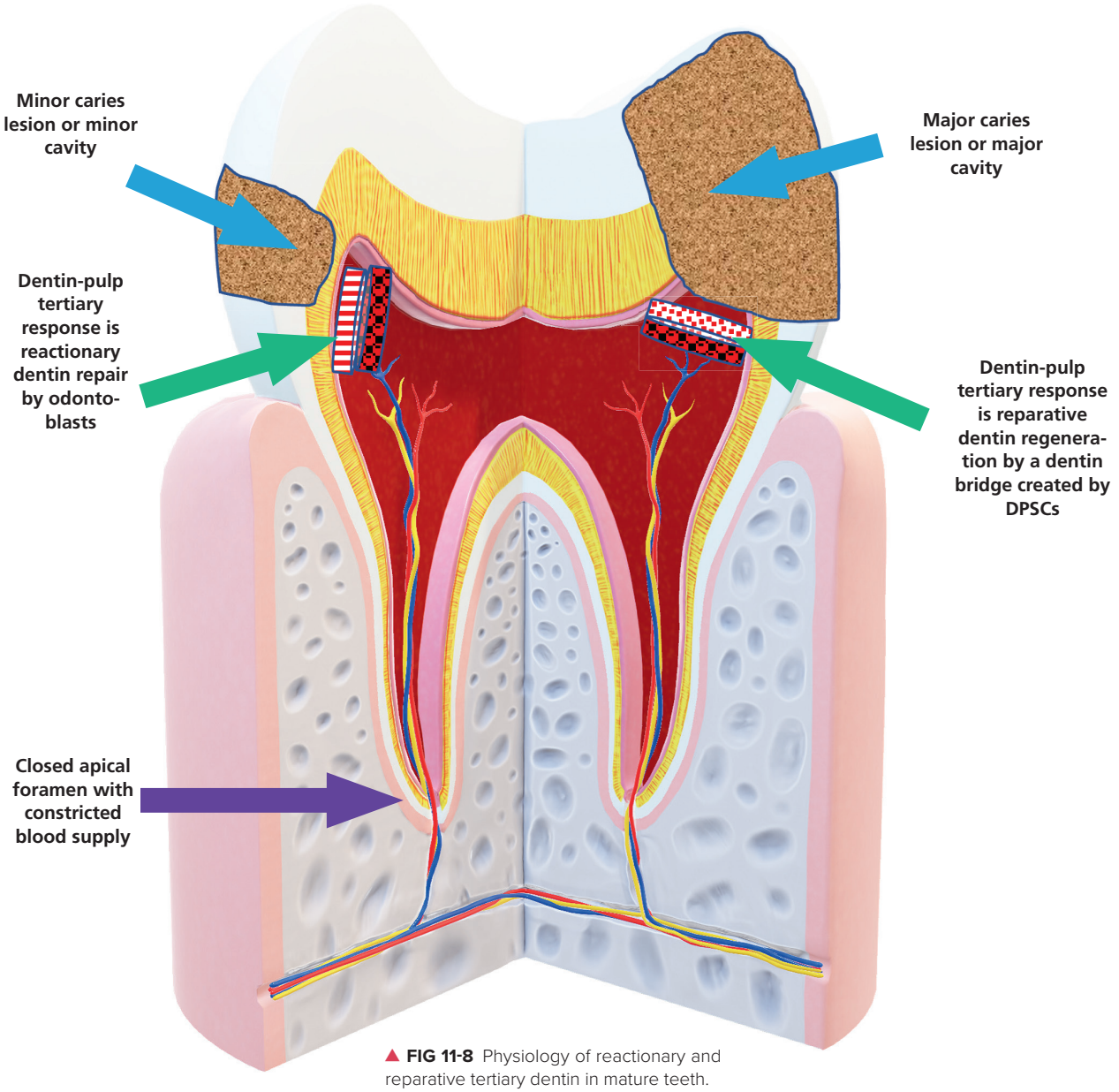



▶ **FIG 11-7** *(a)* The reparative regenerative response to dentin-pulp injury is accomplished by vital DPSCs forming a dentin bridge in the absence of infection and necrosis. 1 = reparative dentin bridge formed across the site of a pulp exposure; 2 = DPSCs create odontoblast-like cells to synthesize the dentin bride to regenerate the tooth and seal the site of the exposed pulp; 3 = vital dental pulp without pulpitis. *(b)* In the presence of a bacterial infection and/or necrosis, there is no dental pulp healing and no stem cell repair.


Minor caries lesion or minor cavity
Major caries lesion or major cavity
Dentin-pulp tertiary response is reactionary dentin repair by odonto-blasts
Dentin-pulp tertiary response is reparative dentin regenera-tion by a dentin bridge created by DPSCs
Closed apical foramen with constricted blood supply

▲ **FIG 11-8** Physiology of reactionary and reparative tertiary dentin in mature teeth.

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**TABLE 11-2** Responses of dentin-pulp odontoblasts to the depth of cavity preparation

| Cavity depth | Cavity remaining dentin thickness (mm) | Odontoblast survival (%) beneath cavity | DPSC replacement of injured odontoblasts | Type of tertiary dentin formation beneath cavity | Pulpitis |
|---|---|---|---|---|---|
| Shallow | 2.5–5.0 | 86% | No | None | Minimal immune cell activity |
| Deep | 0.5–0.01 | 66% | No | Reactionary dentin formation by odontoblasts | Severe immune cell activity if bacterial infection is present |
| Pulp exposed | < 0.01 | 0% | Yes, but not with pulpitis or a bacterial infection | Reparative dentin bridge by DPSCs, but not with necrosis or bacterial infection | Necrosis and severe immune cell activity if bacterial infection is present |

severe injury will demand greater regeneration for repair. Generally, as the risk of dentin-pulp regeneration complications increases, the potential negative effects of contraindications will increase. The risk of regeneration failure will also increase, until the amount of regeneration required is so extensive that it is critically sized and overwhelms the stem cells' ability to accomplish regeneration. *Critical-size (bone) defects* are defined as those that will not heal spontaneously within a patient's lifetime (Roddy et al). Minimally invasive restorative dentistry should always be used to minimize the amount of dentin-pulp removal and injury to reduce the risk of accidentally exposing the dental pulp. Generally, the depth of dentin between a cavity and the dental pulp, called the *remaining dentin thickness*, should be more than 0.5 mm to avoid dental pulp injury (Stanley). Dentists should make every effort to minimize the development of pulp responses and reduce iatrogenic injuries by avoiding the leakage of sealers and etching and conditioning agents and by avoiding placement of dental restorative materials close to the dental pulp without biocompatible liners (Stanley). The dentin-pulp regenerative responses to the remaining dentin thickness of cavity preparations are shown in Table 11-2.

Vital pulp therapies and direct dental pulp capping attempt to maintain the survival of live dental pulp cells following an accidental pulp exposure. Zinc oxide eugenol, amalgam, and composite resins have some toxicity to dental pulp cells. Most dental materials and sealers are not biocompatible and should not be used for pulp capping. Only biocompatible dental restorative materials should be placed in contact with a vital dental pulp to preserve its vitality, such as MTA (Torabinejad and Parirokh), Biodentine, and calcium hydroxide (Fig 11-9). Controversially, MTA has generally replaced calcium hydroxide as the biocompatible material of first choice for most dentists, because MTA can have a greater success rate for direct pulp capping (Zhu et al). MTA has the drawbacks of a long setting time and poor handling characteristics (Saghiri et al). Bioactive or bioinductive dental materials—which contain nanotechnology to promote improved sealing and margin bonding, antimicrobial agents to combat dental pathogens, and growth factors to stimulate increased mineralization and stem cell regeneration (Kunert and Lukomska-Szymanska)—are needed to further increase the success rate of vital pulp therapies.

The restorative variables of deep cavities with less remaining dentin thickness, pulps exposed to restoration materials, and an absence of coolant during cavity cutting have the greatest potential to destroy the underlying pulp cells. Other variables like bur speed, cavity etching treatments, cavity wall depth, cavity volume, cavity surface area, and cavity restoration material have less potential to create dentin-pulp injury. Dentin-pulp regeneration is most likely to be observed in response to deeper cavity preparation, reduced cavity remaining dentin thickness, an absence of coolant during cavity cutting, greater bur speed, and cavity etching treatments, but not the cavity restoration material (Murray et al 2002b). The dentin-pulp injury and regeneration responses following cavity preparation and restoration are shown in Table 11-3.


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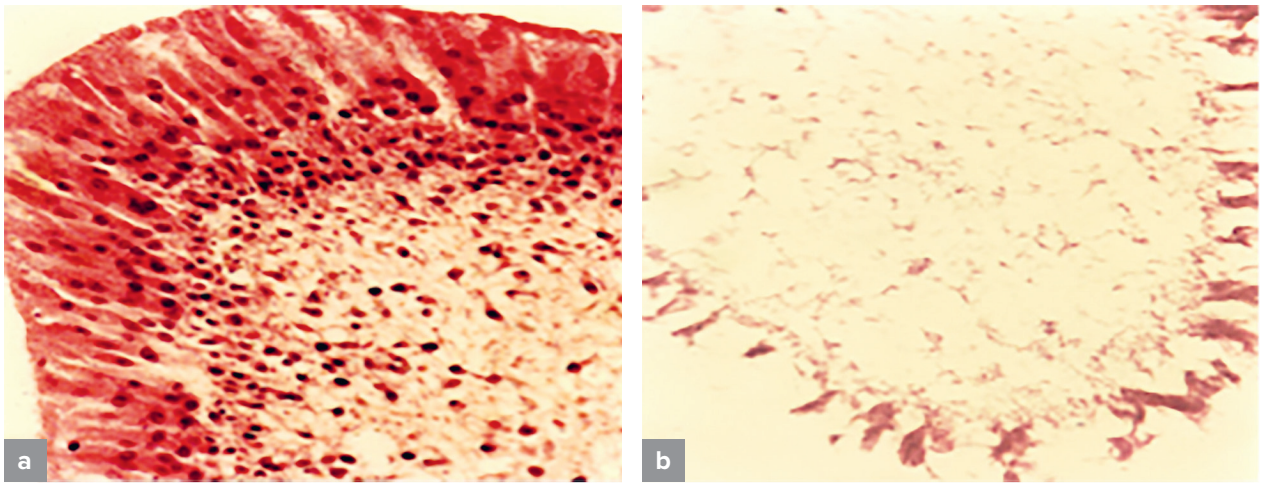


▲ **FIG 11-9** *(a)* Histology of a vital dentin-pulp complex showing intact healthy cells. *(b)* Histology of a nonvital pulp showing necrosis and dead cells following contact with toxic dental materials. The order of toxicity of dental materials when placed in direct contact with the dentin-pulp complex (from least to most toxic) is MTA, composite resins, calcium hydroxide, and zinc oxide eugenol. The most appropriate direct pulp capping materials are biocompatible MTA and calcium hydroxide; while calcium hydroxide can cause superficial necrosis, it can also promote dentin mineralization.

**TABLE 11-3** Dentin-pulp injury and regeneration responses following cavity preparation and restoration

| Variable | Dentin-pulp injury (increased dentinogenesis) | Dentin-pulp regeneration (reparative dentin bridge) |
|---|---|---|
| Cavity remaining dentin thickness | + | + |
| Type of pulp exposed to restoration materials | + | – |
| Absence of coolant during cutting | + | + |
| Bur speed during cavity cutting | – | + |
| Cavity etching treatments | – | + |
| Cavity wall depth | – | + |
| Cavity volume | – | + |
| Cavity surface area | – | + |
| Cavity restoration material | – | – |

+ = has a significant impact; – = has a small impact or no impact.

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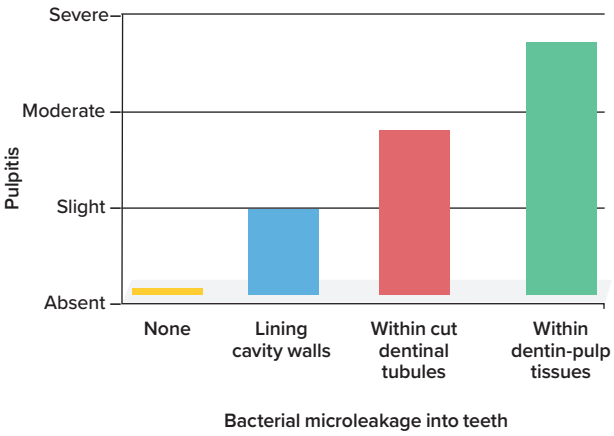
## Bacterial Microleakage and Pulpitis

Bacterial microleakage causes recurrent dental caries, severe pulpitis, and necrosis of the dental pulp, as bacteria and their waste products invade the dentinal tubules, cavity walls, and dentin-pulp complex (Daghrery et al). Dental restorative materials have variable ability to prevent bacterial microleakage; the order from best to worst is as follows: resin modified glass ionomer (100%), bonded amalgam (88%), zinc oxide eugenol (86%), composite resin (80%), gutta-percha (64%), calcium hydroxide (58%), compomer (42%), silicate (36%) and zinc phosphate (0%) (Murray et al 2002a). Etching for binding composite resins to dentin margin was found to be correlated to bacterial microleakage, tertiary dentin formation, and pulpitis. The frequency of bacterial microleakage was as follows: no etching (65%), ethylenediaminetetraacetic acid (EDTA) (51%), and phosphoric acid (20%) (Murray et al 2002a). The correlation between the severity of dental pulpitis and the ingress of bacterial microleakage into the dentinal tubules, cavity walls, and dentin-pulp complex is shown in Fig 11-10.




▲ **FIG 11-10** Bacteria stimulates pulpitis, which is correlated with microleakage penetration of dentin and entry into the dentin-pulp complex. When no bacterial microleakage is observed, pulpitis is absent. Once bacterial microleakage is observed lining the cavity walls, slight pulpitis is observed. When bacterial microleakage is observed within the cut dentinal tubules, moderate pulpitis is observed. And after bacterial microleakage is observed within the dentin-pulp complex, severe pulpitis is observed.

## Saving Traumatized Immature Permanent Teeth of Young Patients

Pediatric dentists and endodontists can now save the traumatized immature permanent teeth of young patients using personalized regenerative treatment strategies (Murray et al 2007), similar to the single- and multiple-visit endodontic steps shown in Fig 11-11.

Toothache often prompts patients to decide to have a painful tooth extracted (Silva-Junior et al), without full consideration given to the impact of the missing tooth in terms of quality of life and the ability to chew food (Hugo et al). Dental implants, bridges, and dentures are esthetically and functionally valuable to replace missing teeth, but they all have disadvantages for patients (Clark and Levin). Ultimately, providing patients with the highest-quality and longest-lasting care requires the accurate differential diagnosis of the dentin-pulp vitality and any irreversible pulpitis and necrosis, followed by adhering to the most appropriate dental inclusion criteria and treatment plan to accomplish dentin-pulp regeneration.

### Dentin-pulp regeneration to save traumatized and diseased immature teeth

One-third of adults have experienced traumatic dental injuries to their permanent dentition, mostly before 19 years of age (Levin et al). Most patients with a fully mature injured tooth will benefit from traditional nonsurgical root canal treatments because of the long-term success rate (over 86%) (Elemam and Pretty). Young children and teenagers with fragile immature teeth can have thin and fragile dentinal walls, which are prone to loss following a stress-overload fracture (Lawley et al). Regenerative endodontic treatment can be beneficial to regenerate a replacement de novo dentin-pulp–like tissue in a disinfected empty root canal (Fig 11-12).

Root canal revascularization by an adequate blood flow through the open root apex is a critical step for the success of regenerative endodontics. The steps to accomplish regeneration of the dentin-pulp complex are shown in Box 11-2.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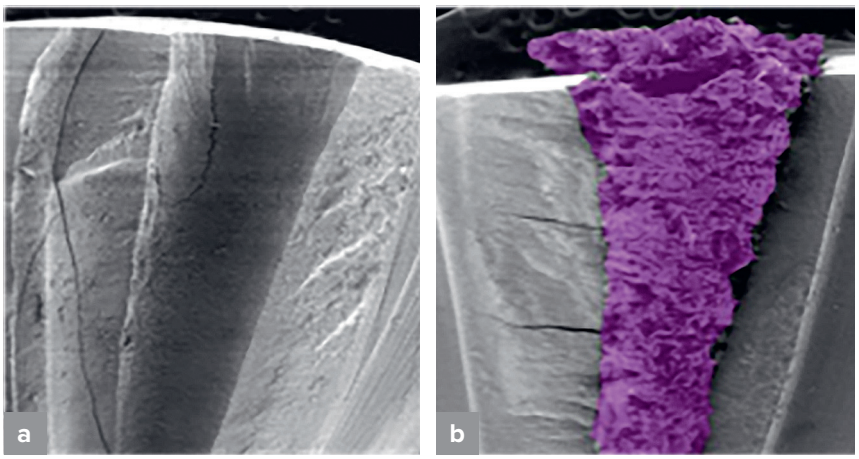
Immature necrotic teeth with thin walls and open apex
Single visit
Multiple visits
Remove necrotic tissue by copious irrigation and files, avoiding the dentinal walls
Irrigate, extricate necrotic tissue, and disinfect by placing antibiotic paste for 1 month
Revascularize root canal by inducing bleeding through root apex from periapical tissues
Add collagen scaffold
No scaffold
Add PRP or PRF from blood
Monitor outcomes

▲ **FIG 11-11** Dentin-pulp endodontic regenerative treatment strategies for immature necrotic teeth. A multiple-visit treatment plan involves the placement of antibiotic paste in the root canal for 1 month to disinfect the canal(s).

▶ **FIG 11-12** Regenerative endodontics showing an empty root canal *(a)* and a regenerated dentin-pulp complex *(b)*.

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**BOX 11-2 Steps to accomplish regeneration of the dentin-pulp complex**

**1. Patient care**

All the case selection criteria for regenerative endodontics must be satisfied.

**2. Tooth selection**

The tooth should be an immature permanent tooth with a wide open apical foramen and an injured or exposed pulp. The dentinal walls must be weak and likely to benefit from continued root development so that they can become stronger and less prone to failure later in life.

**3. Anesthesia**

An anesthetic block of the tooth using 3% mepivacaine is performed without vasoconstrictors (1 carpule every 20 minutes).

**4. Disinfection, irrigation, and instrumentation of the tissues in root canals**

A rubber dam is used to isolate the tooth, and a root canal access wide enough to see all the walls is created. The tooth is then irrigated with 20 mL of 1.25% NaOCl with suction. A hand file and radiograph are used to establish the root canal working length. All necrotic and infected dental pulp is removed, being careful to avoid instrumenting the root canal walls, using a hand or rotary file. The root canals are then packed with a triple antibiotic paste of ciprofloxacin, metronidazole, and minocycline to a final concentration of 0.1 mg/mL, or calcium hydroxide, for up to 4 weeks before the tooth is restored.

**5. Irrigation, chelation, rinsing, and drying of root canals**

The root canals are irrigated with 20 mL of 1.25% NaOCl with suction before being etched with a chelating agent of 17% EDTA or QMix 2in1 (Dentsply Sirona) for 60 seconds. A sterile saline is then used to rinse the root canals, which are dried with paper points.

**6. Revascularization and scaffolds**

Root canal revascularization (bleeding) is induced by stirring the periapical tissue with a bent 3-mm K-file through the root apex, ensuring that blood fills the root canal. A bioabsorbable collagen scaffold is then inserted using a forceps to lightly pack the root canal space from the apex to the cemento-enamel junction.

**7. Tooth restoration**

The root canal opening is sealed with a layer of white MTA, and the tooth is restored with a composite resin restoration. The occlusion is then checked.

**8. Monitoring healing and recall of patient**

The tooth is monitored for flare-ups and periapical lesions during healing, and any retreatment is provided if necessary. The patient should be recalled every 6 months to check for tooth vitality and root development.

Regenerative endodontics can strengthen an immature tooth by continuing the mineralization and maturation of the root dentinal walls, thereby strengthening the root and making it more resistant to fracture. In addition to regenerative endodontics, the treatment options for immature permanent teeth include traditional nonsurgical endodontic root canal treatment, partial pulpotomy, apexogenesis, and apexification.

Clinical regenerative endodontics can fail to revitalize up to 40% of immature teeth (Murray 2018, Wikström et al), suggesting that it may not always be the best choice among all the available endodontic treatments for traumatized or caries-affected immature teeth. In one study, half of the patients had no apparent posttreatment root development or apical closure, and up to a third of the patients had pain or swelling flare-ups following regenerative endodontics (Meschi et al). However, some clinical trials report tooth survival rates as high as 93% (Elfrink et al) and success rates of 80% (Chrepa et al).

The failure of clinical regenerative endodontics may be inevitable in teeth that are not suitable due to patient and tooth selection criteria. Therefore, adherence to the procedural steps as well as to patient- and tooth-qualifying criteria, discussed below, can be extremely important for its success (American Association of Endodontists).

## Selection Criteria for Regenerative Endodontics

Regenerative endodontic treatment outcomes, including for pulp revascularization and vital pulp therapies, can vary widely, with reported success rates of 60% (Wikström et al). The relatively low success rates may be improved by adhering to stricter patient and tooth selection criteria (Fig 11-13), outlined in the following sections.


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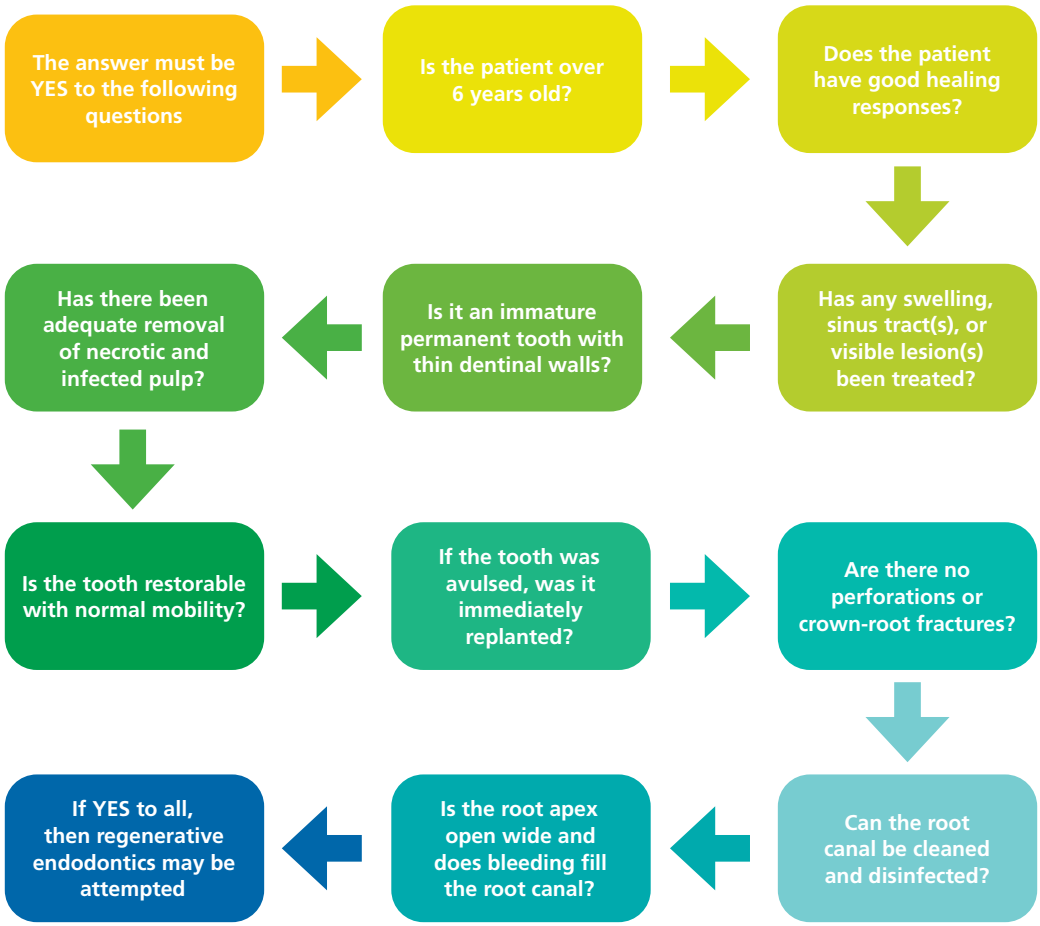
The answer must be YES to the following questions
Is the patient over 6 years old?
Does the patient have good healing responses?
Has any swelling, sinus tract(s), or visible lesion(s) been treated?
Is it an immature permanent tooth with thin dentinal walls?
Has there been adequate removal of necrotic and infected pulp?
Is the tooth restorable with normal mobility?
If the tooth was avulsed, was it immediately replanted?
Are there no perforations or crown-root fractures?
Can the root canal be cleaned and disinfected?
Is the root apex open wide and does bleeding fill the root canal?
If YES to all, then regenerative endodontics may be attempted

▲ **FIG 11-13** Patient inclusion criteria for regenerative endodontic treatment of the dentin-pulp complex.

## Permanent tooth with thin walls in a patient at least 6 years old

Primary teeth should be excluded from regenerative endodontics because of the risk of retaining these teeth and interfering with the eruption pattern of the permanent teeth. Regenerative endodontics should only be provided to permanent teeth, and the minimum age for permanent tooth eruption is 6 years old (Almonaitiene et al). Regenerative endodontics is not beneficial for fully mature teeth with thick dentinal walls, as these teeth do not need root maturation to strengthen their roots.

## Good healing responses

The success of regenerative endodontic treatment depends on revascularization, or the formation of a blood clot within the root canal of the tooth (do Couto et al). This requires that the patient has good healing responses. Patients taking blood-thinning medications (anticoagulants) or who suffer from a systemic disease that can compromise blood clotting have a greater risk of uncontrolled bleeding (Weibert). Uncontrolled bleeding in compromised patients may cause excessive blood loss, tissue damage, and a failure of the blood clot to heal. Thus, medical clearance must be obtained prior to attempting regenerative endodontic treatment in any patient with compromised healing.

## Pretreatment of any swelling, sinus tracts, or visible lesions

Swelling, visible lesions, defects, and sinus tracts associated with the root canal of an immature tooth must be treated and sealed prior to regenerative endodontics to create a long-lasting barrier and prevent microleakage of microorganisms into the root canal. A failure to prevent microleakage and/or a failure to adequately disinfect the

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root canal will compromise healing and will likely result in failure (Wikström et al). Periapical lesions may shrink in size following regenerative endodontic treatment but are unlikely to be completely healed (Kandemir Demirci et al).

### Adequate removal of necrotic and infected dentin-pulp tissues

Regenerative endodontic treatment requires the adequate removal of necrotic and infected dentin-pulp tissues and the disinfection of the root canal prior to root canal revascularization. If there is any persisting bacterial infection or necrotic tissue, it can spread through the regenerated dentin-pulp complex and cause the treatment to fail. Disinfection of the root canals can be effectively accomplished by calcium hydroxide or a broad-spectrum triple antibiotic paste containing ciprofloxacin, metronidazole, and minocycline (do Couto et al, Windley et al) to a final concentration of 0.1 mg/mL, placed inside the root canal for 1 to 4 weeks (American Association of Endodontists). Necrotic tissue is irrigated from the root canal using 20 mL of 1.25% NaOCl using hand and rotary files, followed by a flush of 17% EDTA and a rinse of saline.

### Restorable tooth

A tooth may not be restorable following trauma or caries because it has a crown-root fracture (Levin et al). Crown-root fracture is an absolute contraindication for regenerative endodontics.

### Avulsed tooth replanted immediately

An avulsed permanent tooth must be replanted immediately to avoid healing complications (Cho et al). If the tooth to be treated was avulsed and not replanted immediately, failure is likely.

### Open root canal apex and bleeding fills the root canal

Regenerative endodontic treatment will fail if there is not enough revascularization to deliver stem cells, oxygen, nutrients, and bleeding to fill the root canal space (do Couto et al, Namour and Theys, Wigler et al, Wikström et al). In the absence of revascularization, no replacement dentin-pulp complex will be created, and there will be no revitalization of tissues or mineralization of the dentinal walls to continue root development.

## Other Dentin-Pulp Treatments for Immature Teeth

While regenerative endodontics can be the most beneficial treatment to save immature permanent teeth with thin and weak dentinal walls prone to fracture, it is not always appropriate. Immature teeth that are almost fully developed may have strong dentinal walls that are able to resist tooth fracture. Thus, a diagnostic flow chart can be used to select between regenerative endodontics or apexification, apexogenesis, partial pulpotomy, and conventional root canal treatment, as shown in Fig 11-14. These alternative treatments are discussed in the sections that follow.

### Apexification

Apexification is a procedure that debrides, disinfects, and obturates the root canal of immature teeth with a necrotic dentin-pulp complex or irreversible pulpitis. Chronic pulpitis cannot be reversed to heal the infected dentin-pulp tissues; thus, the prognosis is that the pulp will become necrotic (Ricucci et al 2014). Necrosis is the death of the cells of the dental pulp (Yu and Abbott). Apexification requires the obturation of the root canal with calcium hydroxide, MTA, or a bioceramic root repair material to induce a calcified barrier to help save the tooth (Rafter, Shaik et al). A meta-analysis that compared MTA, EndoSequence (Brasseler), and bioceramic root repair materials for apexification revealed that all three of these biomaterials had similar success rates above 90% (Shaik et al), which is a 10% improvement over the 80% reported success rate of calcium hydroxide for apexification (Damle et al). A drawback of using calcium hydroxide for apexification is that it requires multiple appointments to replace the calcium hydroxide, whereas the MTA need only be applied once (Guerrero et al). However, the practical limitations of apexification are that it cannot promote maturation of the immature tooth root and thus cannot increase the thickness of the dentinal walls to strengthen the tooth, making it a poor treatment choice for fragile immature teeth with very thin dentinal walls. However, apexification can be an optimal endodontic treatment for immature teeth with a necrotic pulp where the root dentinal walls are already thick enough to resist a fracture (see Fig 11-14).


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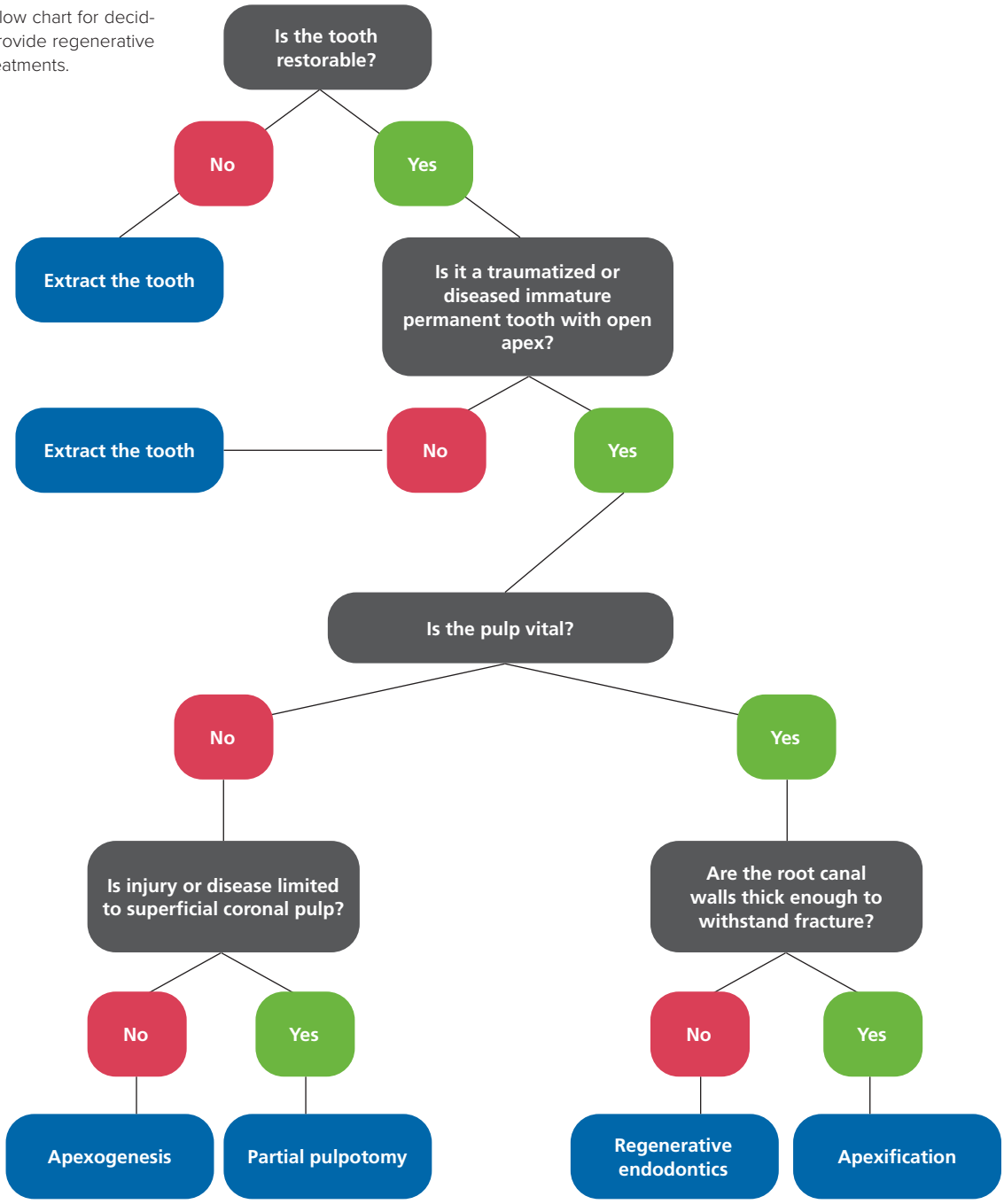


▶ **FIG 11-14** Flow chart for deciding when to provide regenerative dentin-pulp treatments.

## Apexogenesis

Apexogenesis is a vital dentin-pulp procedure that removes the coronal injured pulp from the root canal of an immature permanent tooth. The removal of the superficial injured and/or infected pulp is necessary to prevent the spread of necrosis/infection/irreversible pulpitis to the remaining vital, uninfected, and uninflamed pulp and to continue the maturation and physiologic development of the roots (Goldstein et al, Mousivand et al). Apexogenesis can permit the remaining vital dentin-pulp complex to continue its maturation and strengthen the roots to withstand a potential facture (Goldstein et al, Mousivand et al).

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Apexogenesis is a vital pulp capping treatment, whereas apexification is a nonvital (necrotic pulp) treatment. Apexogenesis maintains the survival of the vital pulp to continue root maturation and thickening, which can strengthen the tooth to resist fracture. Thus, because the pulp is still vital and able to mineralize root dentin, there is also no rational justification for providing a regenerative endodontic treatment or a conventional root canal obturation treatment (see Fig 11-14). The long-term success rate of apexogenesis in anterior teeth and posterior teeth was about 82.5% and 96.4%, respectively (Mousivand et al).

### Traditional nonsurgical root canal treatment

The gold standard treatment for most mature teeth with a painful and/or necrotic pulp is conventional nonsurgical root canal treatment (Nagendrababu et al). It uses instruments, irrigants, and chelating agents to debride infected tissues and disinfects the root canal by cleaning, shaping, sealing, and obturating, thereby saving the natural tooth (Metzger et al, Peters). The long-term survival rates for teeth following nonsurgical endodontic treatment can exceed 86%, and tooth survival after nonsurgical endodontic retreatments can exceed 78% (Elemam and Pretty).

### Partial pulpotomy

Partial pulpotomy is a vital pulp procedure that partially debrides the coronal injured pulp from a root canal and allows the remaining vital pulp to continue its physiologic root maturation, strengthening the roots to protect them from fracture (Mejàre and Cvek, Sari). In partial pulpotomy, 2 mm of coronal injured pulp is debrided from a root canal.

Partial pulpotomy is similar to apexogenesis in that it removes some coronal pulp, leaving a vital healthy pulp in the roots of immature teeth. The only difference is that the amount of debrided tissue is usually fixed at 2 mm for partial pulpotomy, whereas for apexogenesis it can be less or more according to the skill of the clinician and the extent of damage to the immature tooth (severity of trauma, size and position of the caries or fracture) (Shah et al). The long-term healing success of partial pulpotomy can exceed 93% (Mejàre and Cvek).

## Variable Success of Regenerative Endodontics

The clinical success of regenerative endodontic treatments can vary between a tooth survival of 97% to only 20% when radiographic changes are used as an end point (Ong et al). The high variability of the efficacy of regenerative endodontics in the literature may be due to gaps in knowledge (Tong et al) or the lack of a direct comparison of MTA and regenerative endodontic treatment (Torabinejad et al). However, the most likely reason for the variable success of regenerative endodontics is variations among the patient and tooth selection criteria (American Association of Endodontists). The treatment options for failed regenerative endodontic procedures include apexification or repeating the regenerative endodontic procedure. Regenerative endodontic retreatment has been successful (Chaniotis, Nosrat et al).

Regenerative endodontics is uniquely able to continue the root development in immature permanent teeth with a necrotic pulp, thereby potentially saving the teeth for the lifetime of the patient. Conventional endodontic root canal treatment, partial pulpotomy, apexogenesis, and apexification, on the other hand, should always be provided when these treatments are more likely to benefit the patient because they can be more successful than regenerative endodontics (Murray and Garcia-Godoy 2012).

A comparison of blood clot revascularization versus PRP versus PRF discovered that PRP and PRF may be more successful for accomplishing apical closure or decreasing the size of the root apex, inducing periapical healing response and root lengthening (Murray 2018).

The advantage of regenerative endodontics, as an alternative treatment to apexification, is that by continuing root development to thicken and strengthen the canal walls, it can save fragile immature permanent teeth by protecting them against fracture. If regenerative endodontics can prevent the loss of permanent teeth in children and young adults, it can avoid their lifelong need for dental implants, bridges, or dentures. Furthermore, disinfection of the root canal can heal periapical lesions and continue root apex development, and regeneration of the vitality of the dentin-pulp complex can restore sensitivity to teeth.

The limitation of regenerative endodontics is that it does not benefit fully mature teeth with a closed root apex, because these mature teeth likely have thick dentinal walls to withstand fracture. Regenerative endodontics therefore should not be provided to fully mature teeth with a closed root apex, because it is not likely to be more beneficial than a traditional nonsurgical endodontic treatment. Regenerative endodontics also should not be provided to teeth with a vital dentin-pulp complex, without pulp infection, and without deep caries, because apexogenesis is likely to be a more successful treatment. Regenerative endodontics should not be attempted in teeth with a complicated root canal morphology that would prevent the adequate disinfection and removal of necrotic tissues. Failure to


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adequately disinfect the root canal will risk spreading the infection and necrosis to adjacent tissues. In addition, regenerative endodontics will fail if the white MTA or intracanal barrier biomaterial is obturated below the level of the cementoenamel junction (CEJ), because it will prevent dentin regeneration at that weak CEJ area, where the tooth is likely to fracture. Retreatments for failed regenerative endodontics are challenging (Lee and Song) because the spread of recurrent necrosis and bacterial infection is likely to be worse and therefore even more difficult to debride and disinfect adequately to allow stem cell dentin-pulp regeneration.

## Conclusion

Astoundingly, cloning teeth in rodents, tooth banking, genetic cell reprogramming, using regenerative endodontics to revitalize a tooth with replacement dentin-pulp tissue, and harvesting stem cells or scaffolds to regenerate missing tissues have already become a reality. Minimally invasive dental restorative treatments can help reduce the risks of an accidental pulp exposure and subsequent dentin-pulp injury. While regenerative endodontics certainly holds promise for saving more teeth, these therapies are not always the most likely to benefit patients with traumatized immature permanent teeth, and the suitability of apexification, apexogenesis, partial pulpotomy, and conventional root canal treatment must always be considered during treatment planning.

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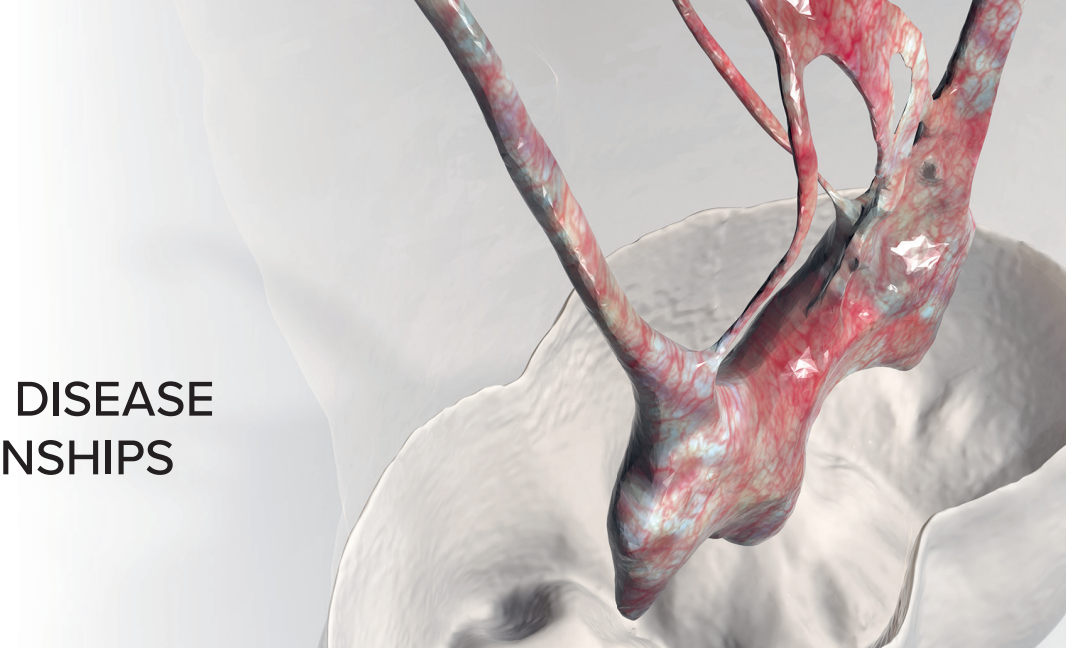
# 12

# PULPAL AND PERIODONTAL DISEASE INTERRELATIONSHIPS

Dustin Weitz
James D. Johnson
Scott B. McClanahan



The tissues of the dental pulp and the periodontium are intimately linked through their embryologic origins and their anatomical and vascular relationships. These relationships are significant in both health and disease. Disease entities often challenge the clinician in differential diagnosis, sequencing of treatment, and determining the prognosis. Microorganisms remain the primary etiologic factor in conjunction with contributing factors such as cracks and fractures, poor endodontic treatment, poor restorative treatment, perforations, trauma, root resorption, cemental tears, and developmental irregularities that directly impact the initiation and pathogenesis of disease processes. Endodontic and periodontal diseases are multifactorial and depend on specific etiologic factors for the progression of disease. The astute clinician will strive to understand these interrelationships to determine an accurate diagnosis, recommend an appropriate treatment plan, and improve the prognosis for the patient.

## Pathways of Communication and Anatomical Relationships

The dental pulp and the periodontium are linked by several pathways of communication and anatomical relationships: *(1)* exposed dentinal tubules, *(2)* lateral and accessory canals, *(3)* the apical foramen, and *(4)* developmental anomalies.

### Dentinal tubules

Microorganisms and their byproducts cannot penetrate through intact cementum but are able to penetrate dentin. Therefore, exposed and patent dentinal tubules provide a pathway for pathogens between the pulp and the periodontal ligament (Ehnevid et al 1995, Das et al). Tubules become exposed by several mechanisms such as periodontal disease, developmental defects, and periodontal or surgical procedures (Shambarger et al). Additionally, there is an anatomical arrangement between enamel and cementum where tissues do not meet or overlap but have a gap, which leaves dentinal tubules exposed (Neuvald and Consolaro).

Mjör and Nordahl reported that dentinal tubule size varies from 1 to 3 μm in diameter. An extreme range in the tubule density was found—from 7 to 30 tubules per 100/mm or 4900 to 90,000 tubules per $mm^2$. The number of tubules per 100 mm in the peripheral dentin was found to be relatively constant except for higher density in the cusps.

Dentinal hypersensitivity can be caused by fluid movement in patent dentinal tubules, as shown by the hydrodynamic theory (Brännström and Aström). Without intact enamel or cementum protection, the pulp can become exposed to the oral environment via patent dentinal tubules in the gingival sulcus, a periodontal pocket, or when there is gingival recession. Bergenholtz and Lindhe (1975) demonstrated that soluble material from bacterial plaque applied to exposed dentin could cause pulpal inflammation, which indicates the significance of this pathway between the periodontal tissues and the pulp. The number of patent tubules in a given area was significantly greater in sensitive teeth than nonsensitive teeth, and the tubules were about twice as wide in sensitive teeth (Absi et al).

### Lateral and accessory canals

Ramifications of the root canal system, lateral and accessory canals can be found at any place along the length of the root but are mainly located in the apical third (Rubach and Mitchell). De Deus reported that an average of 27.4%

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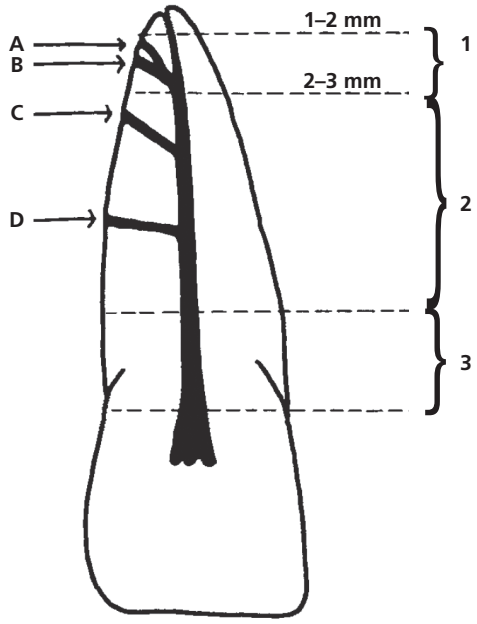


▲ **FIG 12-1** Accessory (A), secondary (B), and lateral (C and D) canals in the apex (1), body of the root (2), and base of the root (3). (Reprinted with permission from De Deus.)

of human teeth demonstrated some canal ramification, with 1.6% located in the coronal third, 8.8% in the middle third, and 17% in the apical third (Fig 12-1). In a study of maxillary molars, Degerness and Bowles found that 80% of the accessory canals were found in the apical 3.64 mm. However, it seems that the incidence of periodontal disease associated with these types of canals is relatively low. In a study of human teeth extracted due to periodontal disease, Kirkham reported that only 2% of accessory canals were associated within a periodontal pocket.

Furcation canals represent types of accessory canals that are in the furcation of posterior teeth. Gutmann described the "furcation region" of molar teeth as the furcation plus 4 mm down the internal surface of the root. A number of studies using a variety of methodologies have evaluated the prevalence of furcation canals or a patent furcation region and demonstrate quite a wide range of findings, from 0% to 86.5% (Lowman et al, Vertucci and Williams, Hession, Vertucci and Anthony, Shambarger et al). Furcation canals provide a potential pathway of communication between the pulp and periodontal tissues, which becomes important when periodontal disease exposes the furcation region or when pulpal inflammation impacts the health of the tissues in the furcation.

### Apical foramen

The major pathway of communication between the pulp and the periodontium is the apical foramen. Microorganisms and their proinflammatory mediators in the pulp have a direct pathway through the apical foramen to the apical tissues to be able to cause periradicular disease with its accompanying bone loss and resorption of roots (Seltzer et al 1963). Periodontal disease with associated deep pocketing that reaches the apical foramen causes substantial pathologic pulpal changes (Langeland et al, Ricucci et al 2021).

### Developmental anomalies

Palatoradicular grooves have an incidence of 4.6% in maxillary lateral and central incisors, and 54% of the grooves terminate in root structure (Kogon). Withers et al found a prevalence of 8.5%, with 93.8% occurring in maxillary lateral incisors. Lara et al found that the grooves terminated in the middle third of the root at a rate of 69%, with the remaining 31% in the apical third. These anomalies are difficult for the patient to maintain and may require interdisciplinary treatment (Cho et al) or surgical intervention (Garrido et al).

Cervical enamel projections are enamel projections from the cementoenamel junction that dip toward the furcal area, with an occurrence rate of 82.5% in molars with furcation involvements. Surgery with enameloplasty promoted a more cleansable area for patients (Hou and Tsai).

## Pathways of Communication and Disease Relationships

Pathways of communication facilitate the biologic relationship between the pulp and periodontal tissues. The specific aspects of the influence of this relationship have been debated extensively.

Pathologic conditions in the pulp cause inflammatory change in the periodontium relatively quickly, whereas inflammation and pathologic conditions in the periodontium cause degenerative change in the pulp gradually (Sinai and Soltanoff). Inflamed or necrotic pulps swiftly spread through lateral and accessory canals to cause inflammation of the periodontal tissues (Seltzer et al 1963). Rubach and Mitchell reported that pulpal inflammation could occur from periodontitis via accessory canals.

Mazur and Massler found no relationship between severity of periodontal disease and changes in the pulp. Czarnecki and Schilder and Torabinejad and Kiger also observed no association between the severity of the periodontal disease and pulpal changes. Langeland et al described that periodontal disease impacted the pulp through inflammation, calcification, and resorption, but the pulp did not succumb until the apical foramen was involved. Teeth exposed to periodontitis demonstrated mild pathologic pulp tissue alterations (57%), and


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periodontal destruction limited to the cervical half of the root did not cause severe changes in the pulp (Bergenholtz and Lindhe 1978). In cases of periodontal pocket formation from periodontal disease, Ricucci et al (2021) reported that the pulpal reaction was nominal.

Untreated endodontic infection, demonstrated by periapical radiolucency, may interfere with periodontal healing after periodontal therapy (Ehnevid et al 1993) and should be considered a risk factor in the progression of periodontal disease (Jansson et al 1993). Teeth with persisting or progressing periapical pathosis were found to lose comparatively more radiographic attachment than teeth with no signs of periapical pathosis (Jansson et al 1995a).

Endodontic therapy or pathosis had no apparent influence on marginal bone levels in patients with minimal to no periodontal disease (Miyashita et al). Endodontic infection in mandibular molars was associated with attachment loss in furcation areas and can be a risk factor influencing the prognosis of molars in patients prone to periodontal disease (Jansson and Ehnevid). A positive relationship was found between the severity of periodontal disease and histologic alterations in the dental pulp, where more attachment loss was associated with pathologic transformation in the pulp (Wan et al). In teeth not responding to periodontal therapy, chemomechanical preparation and intracanal medication with calcium hydroxide reduced infectious content in both the pulp canal space and the periodontal pocket (Louzada et al).

In teeth with advanced periodontal disease, pulpal necrosis developed with a significantly higher rate in abutment teeth than in non-abutment teeth (Bergenholtz and Nyman). The impact of caries, restorative procedures, and periodontal disease is cumulative, and over time the ability of the pulp to recover from insult diminishes (Solomon et al). Pulpal clinical involvement with no response to pulp testing occurred only in the most advanced stage of chronic periodontal disease with apical involvement (Neves et al).

## Etiology of the Disease Processes

Microorganisms are etiologic factors for both endodontic and periodontal diseases (Kakehashi et al, Möller et al, Bergenholtz, Lin et al 2006, Ricucci et al 2006, Curtis et al 2005, Van Winkelhoff and Boutaga, Vitkov et al). Periodontitis is a polymicrobial infection (Moore, Socransky et al), and heredity and local factors such as smoking influence the occurrence and severity of the disease (Page et al). The microbial ecosystem in periodontal disease is complex and dynamic (Socransky et al, Costalonga and Herzberg, Vieira Colombo et al, Curtis et al 2020).

Endodontic disease is also a polymicrobial infection (Fabricius et al, Siqueira and Rocas 2009b, Chugal et al), and the concept of biofilms has become extremely important in understanding the pathogenesis of endodontic disease and persistent infections (Leonardo et al, Chavez de Paz).

Dental plaque is a common example of a biofilm. Biofilm formation gives bacteria the ability to rapidly adapt to their environment, and the complexity of this microenvironment enables its inhabitants to become established and proliferate in inhospitable surroundings (Hall-Stoodley et al, Siqueira and Rocas 2009a).

## Contributing Factors

The factors contributing to combined and concomitant endodontic-periodontal lesions include cracked teeth, vertical root fractures, cemental tears, poor endodontic treatment, poor restorations, iatrogenic and resorptive perforations, and trauma. Often the disease condition is not exclusive to any one of these contributors but rather may be multifactorial, and the clinician must be able to identify and appropriately address all contributing aspects to achieve a favorable outcome.

### Cracks and fractures

Incomplete longitudinal fractures in teeth are frequently encountered in the practice of dentistry and endodontics. Depending on the depth of a crack, it can lead to endodontic disease, periodontitis, or both. It is critical that clinicians carefully evaluate for fractures when treating endodontic and periodontal lesions. In teeth with cracks identified early, a pulpal diagnosis of symptomatic reversible pulpitis, and interproximal periodontal pocket depths of 3 mm or less, treatment with full-coverage restorations is predictable and successful. However, approximately 20% of these teeth will require root canal treatment within 6 months, which is only slightly higher than the reported incidence for teeth needing root canal treatment after crown placement whether a crack is present or not (Krell and Rivera).

As longitudinal cracks progress, the pulp and periodontium become involved, and differentiating between interproximal bone loss associated with a crack and that from periodontal disease can be difficult. In a study examining the 12-month survival rate of 2,086 cracked teeth receiving orthograde endodontic treatment, an overall success rate of 82% was found. If there was a mesial or distal periodontal probing depth of 5 mm or greater, the success rate fell to only 41%. Other factors that adversely affected the success rates were crack lines through the distal marginal

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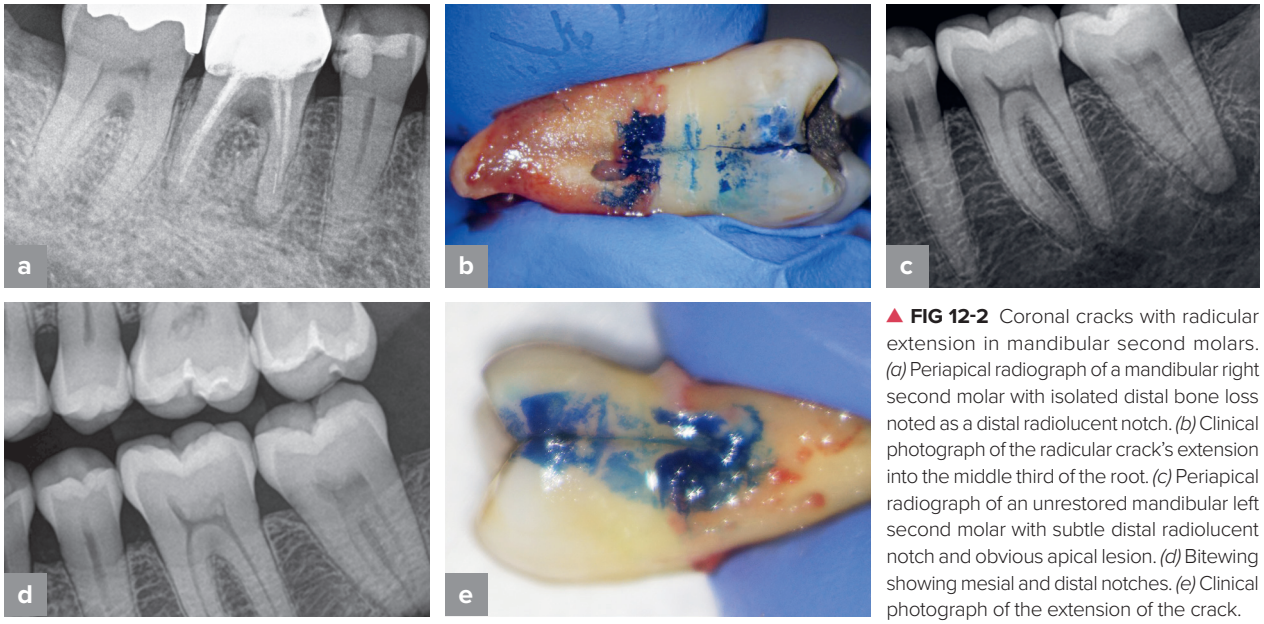
ridge and periapical diagnoses of chronic apical abscess, symptomatic apical periodontitis, or acute apical abscess (Krell and Caplan). Kang et al noted a similar decrease in the survival of cracked teeth, with the 2-year survival rate dropping from 97% to 74% if a pocket depth of 6 mm or greater was present; mandibular second molars were the teeth most often associated with cracks. Figure 12-2 demonstrates two different mandibular second molars with coronal cracks that extend to the middle third of the root. When a coronal crack is the suspected cause of pulpal, periodontal, or periapical disease, it is prudent to stain the internal aspect of the access preparation and pulp chamber and inspect under high magnification to determine the extent of the fracture. Figure 12-3 shows a maxillary left first molar with obvious periodontal and periapical bone loss and a mandibular right second molar with subtle radiographic evidence of periodontal and periapical pathosis.

Catastrophic fractures can occur in unrestored teeth and lead to fracture necrosis. With fracture necrosis, a mesial to distal fracture extending below the level of the alveolar crest is typically present. The subsequent pulpal necrosis can cause significant apical bone loss, while the pathogens present within the crack can cause extensive periodontal destruction, often necessitating extraction as the only treatment option (Berman and Kuttler). Figure 12-4 illustrates the classic radiographic presentation of fracture necrosis of the mandibular left first molar, with the CBCT showing an isolated low-density area consistent with bone loss adjacent to the radicular extension of the fracture and the periapical radiograph showing a distal radiolucent notch and periapical radiolucency consistent with apical periodontitis or apical abscess.

There is also evidence that teeth with coronal fractures with radicular extension can be maintained with the proper treatment protocol. In a prospective clinical study, Davis and Shariff found a 90.6% success rate and 96.6% survival rate at 2 to 4 years for endodontically treated teeth with cracks with radicular extensions. The treatment protocol included 2 to 3 weeks of intracanal medicament with aqueous calcium hydroxide if a periradicular radiolucency was present, removal of gutta-percha 2 to 3 mm apical to the crack, and restoration with a bonded fluoride-releasing resin into the canals and over the pulpal floor. The teeth were taken out of occlusion, and the patients were instructed to avoid chewing in the area and to get a full-coverage restoration fabricated and placed as soon as possible, followed by a 6-week posttreatment evaluation to adjust the occlusion.

Vertical root fractures are a completely different entity than cracked teeth and are almost exclusively seen in teeth with prior endodontic treatment, where the fracture initiates in the root and progresses coronally, most often with a buccolingual orientation. Cracks, on the other hand, are typically oriented in a mesiodistal direction, initiate in the crown of the tooth, and progress apically (Rivera and Walton). A sinus tract can be present, and once a vertical root fracture has reached the coronal third of the root, often there are isolated and deep buccal or lingual periodontal pockets present adjacent to the fracture, which can present




▲ **FIG 12-2** Coronal cracks with radicular extension in mandibular second molars. *(a)* Periapical radiograph of a mandibular right second molar with isolated distal bone loss noted as a distal radiolucent notch. *(b)* Clinical photograph of the radicular crack's extension into the middle third of the root. *(c)* Periapical radiograph of an unrestored mandibular left second molar with subtle distal radiolucent notch and obvious apical lesion. *(d)* Bitewing showing mesial and distal notches. *(e)* Clinical photograph of the extension of the crack.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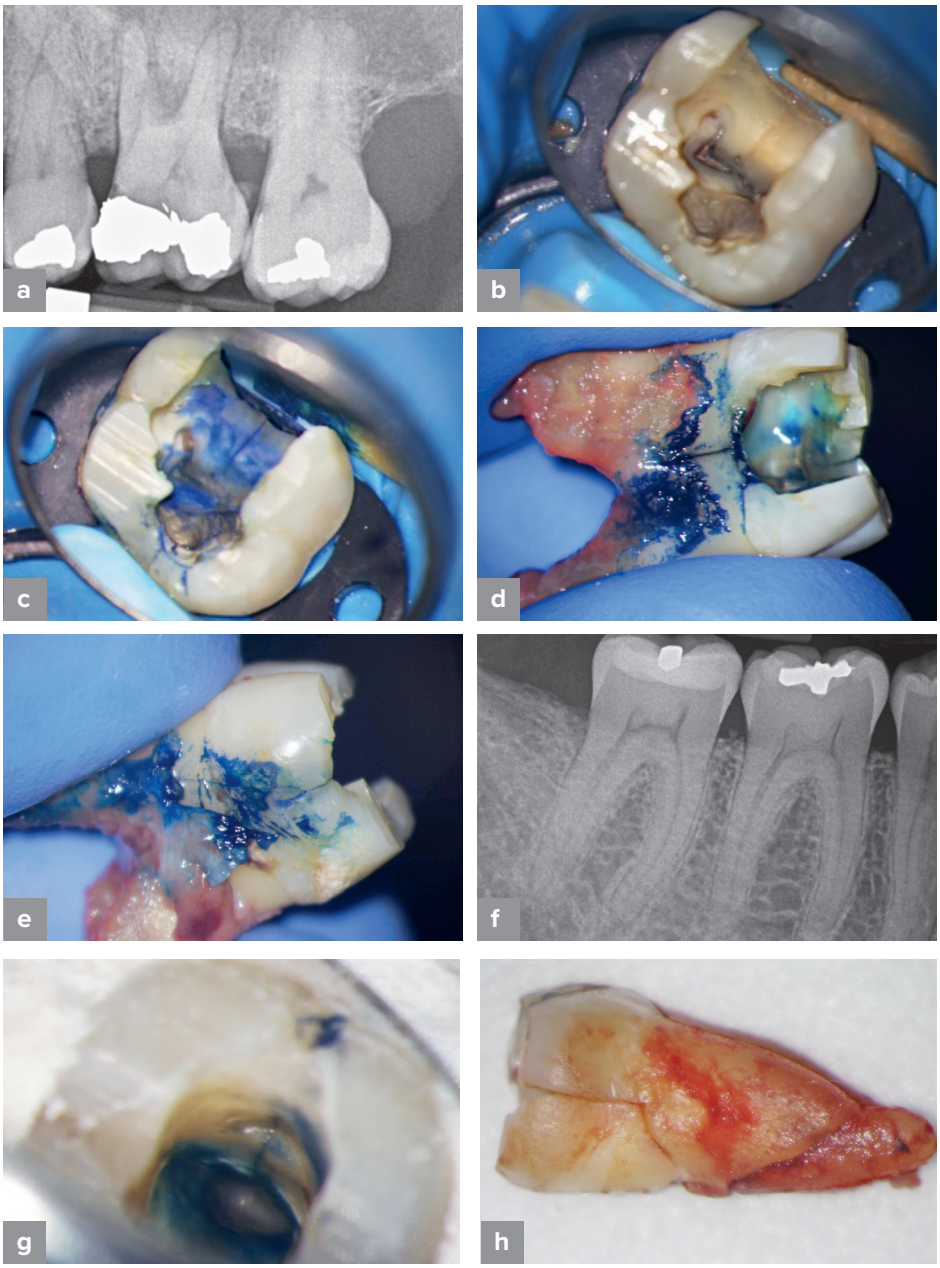


▲ **FIG 12-3** *(a to e)* Coronal crack in a maxillary left first molar with radicular extension. *(f to h)* Mandibular right second molar with coronal crack through the distal marginal ridge with radicular extension.

similar to a chronic apical abscess that is draining via the periodontal ligament space such as an endodontic-periodontal lesion of primary endodontic origin (Tamse). The prognosis for treatment is unfavorable, with extraction as the most common treatment. Figure 12-5 shows a classic vertical root fracture of the mandibular right second molar with prior endodontic treatment and a buccal to lingual fracture of the mesial root.

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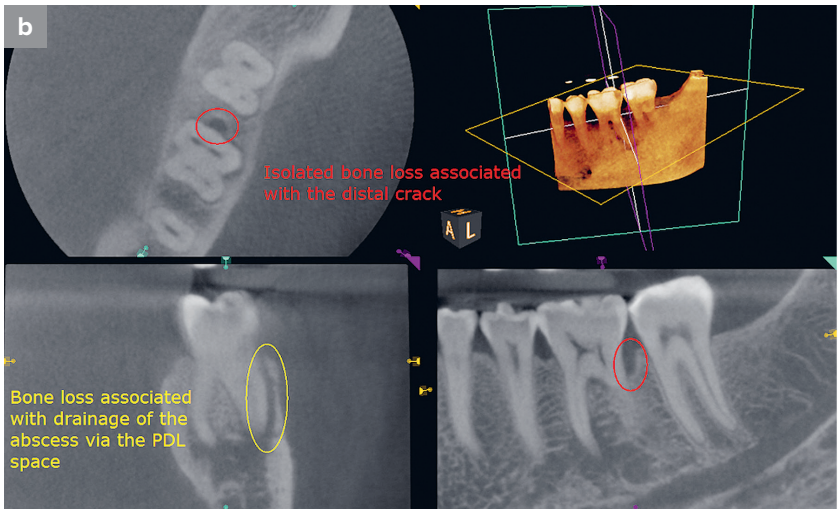
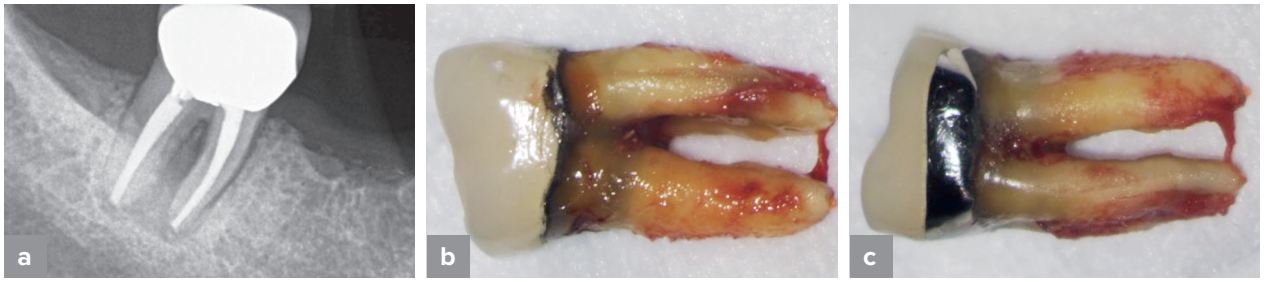

b
Isolated bone loss associated with the distal crack
A L
Bone loss associated with drainage of the abscess via the PDL space

◀ **FIG 12-4** Pulpal necrosis in a mandibular left first molar. *(a)* Distal radiolucent notch and periapical radiolucency. *(b)* CBCT with a distal semicircular low-density area from the alveolar crest to the middle third of the root.




▲ **FIG 12-5** Vertical root fracture. *(a)* Periapical radiograph of the mandibular right second molar showing furcal bone loss. *(b and c)* Clinical photographs showing the progression of the fracture and the root surface loss of viable periodontal ligament adjacent to the fracture lines.

## Cemental tears

A *cemental tear* is a unique type of root fracture that Watanabe et al define as a complete or incomplete separation of cementum from the cementodentinal junction or along an incremental cementum deposition line within the body of the cementum but that rarely involves fracture of the dentin. Depending on where the detachment of the cementum occurs, the associated periodontitis can mimic periodontal disease, a lesion of endodontic origin, or a combination endodontic-periodontal lesion, often with severe periradicular bone loss. An accurate diagnosis is critical for appropriate and successful treatment. Several clinical features and predisposing factors have been suggested and may be of help when identifying cemental tears. Lin et al (2011) found that age, sex, tooth type, and attrition are predisposing factors for cemental tears; maxillary and mandibular incisors were the most common teeth affected, with a predilection for male patients and patients over the age of 60 years. The size and position on the root of cemental tears can be highly variable, leading to multiple modalities of treatment. Lee et al (2021) proposed a staging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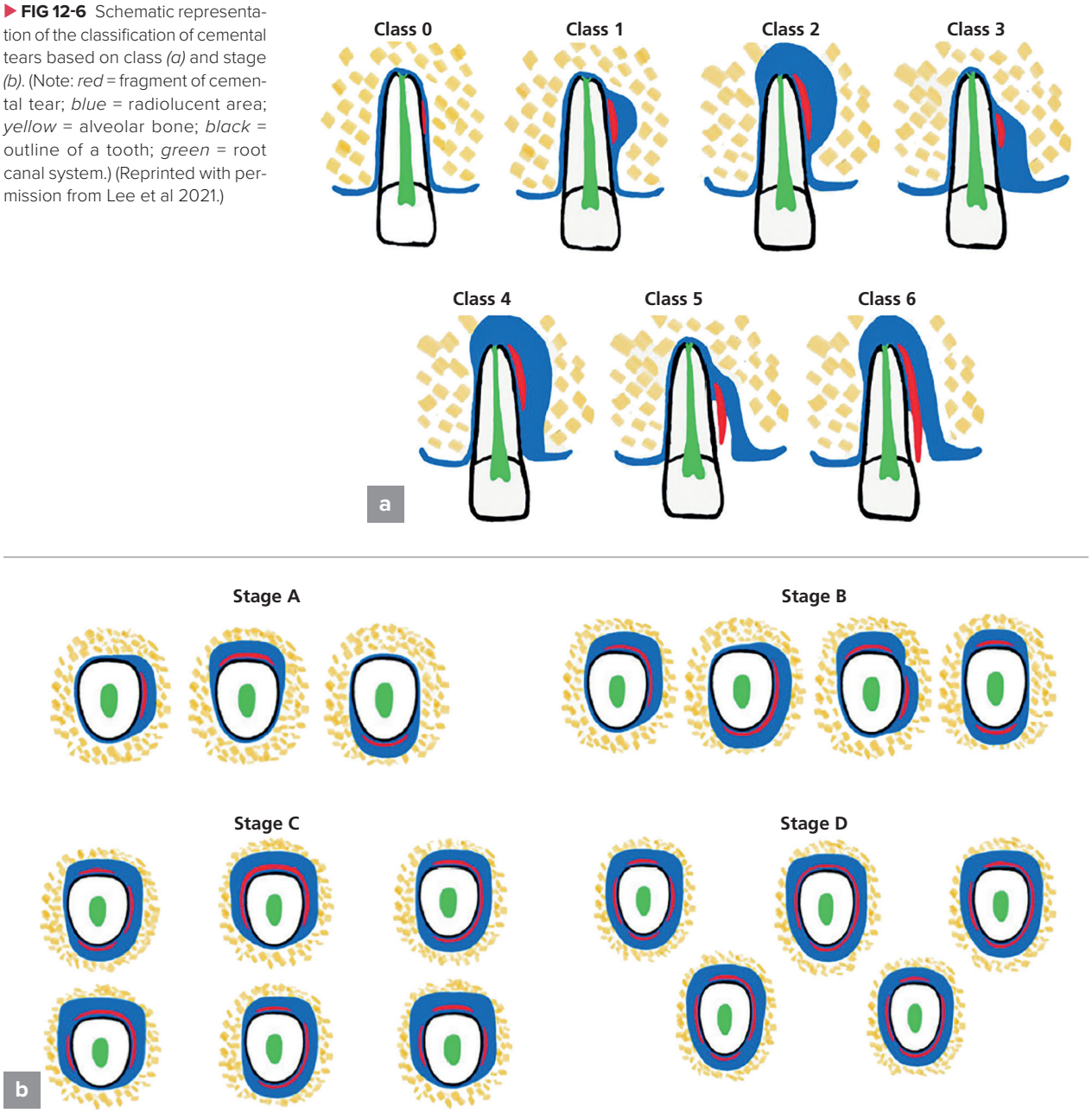


**FIG 12-6** Schematic representation of the classification of cemental tears based on class *(a)* and stage *(b)*. (Note: *red* = fragment of cemental tear; *blue* = radiolucent area; *yellow* = alveolar bone; *black* = outline of a tooth; *green* = root canal system.) (Reprinted with permission from Lee et al 2021.)

and classification system for cemental tears that can aid in diagnosis and treatment planning, help to evaluate prognosis for treatment, and improve communication among treatment providers (Fig 12-6).

There are three components of successful cemental tear treatment: *(1)* removal of the detached cemental fragment, *(2)* resolution of the periodontal and/or endodontic pathoses, and *(3)* reestablishment of healthy periodontal soft and bony tissues. Interdisciplinary treatment can involve nonsurgical endodontic treatment or retreatment, occlusal adjustment and splinting, nonsurgical periodontal treatment, surgical periodontal treatment (possibly with root-end resection and root-end filling), guided regenerative treatment, or extraction (Lee et al 2021). Figure 12-7 shows extensive periodontal bone loss associated with a cemental tear on the distal aspect of a mandibular left second molar. Figure 12-8 illustrates the case of a cemental tear causing periodontal bone loss in the maxillary right central incisor. Figure 12-9 shows the diagnosis and treatment of an apical cemental tear mimicking apical periodontitis of endodontic origin.

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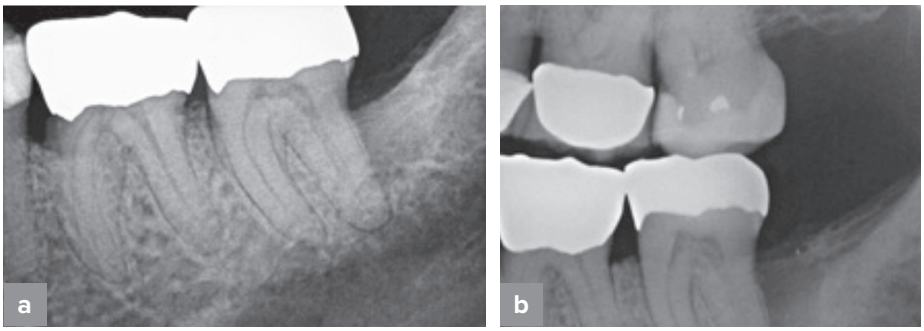
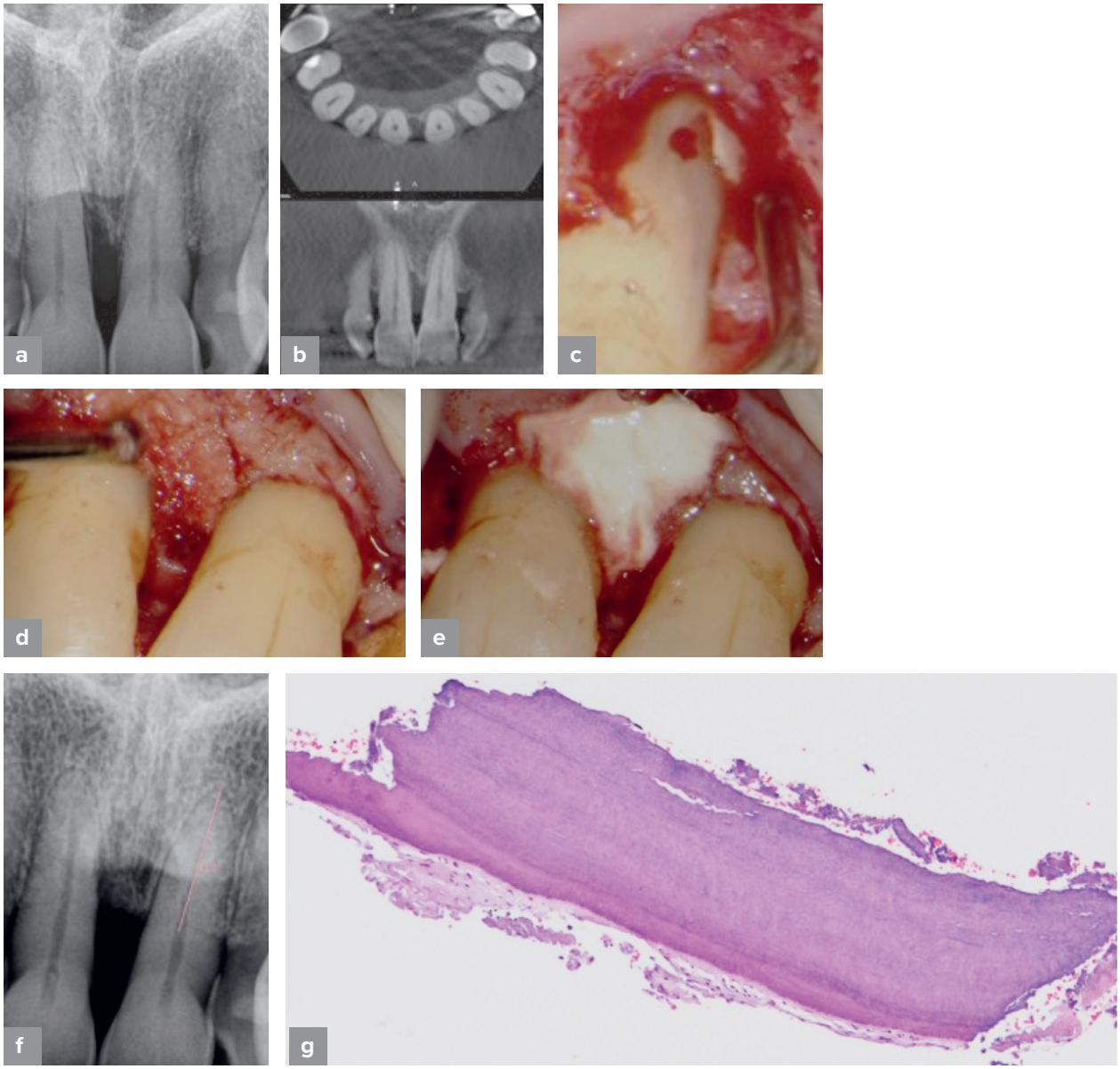



◀ **FIG 12-7** Cemental tear mimicking periodontal disease in the mandibular left second molar. *(a)* Periapical radiograph showing a thin detached fragment of cementum on the distal aspect of the tooth. *(b)* Bitewing showing the periodontal bone loss associated with the cemental tear.




▲ **FIG 12-8** Cemental tear mimicking periodontal disease in the maxillary right central incisor. *(a and b)* Preoperative periapical and CBCT images showing a cemental tear causing a periodontal defect on the mesial aspect of the tooth. *(c)* Clinical photograph identifying the cemental tear. *(d and e)* Placement of a bone graft with calcium sulfate following removal of the tear and application of Emdogain (Straumann). *(f)* Periapical radiograph from 12-month posttreatment evaluation. *(g)* Photomicrograph of tissue obtained for biopsy confirming that the fragment is cementum.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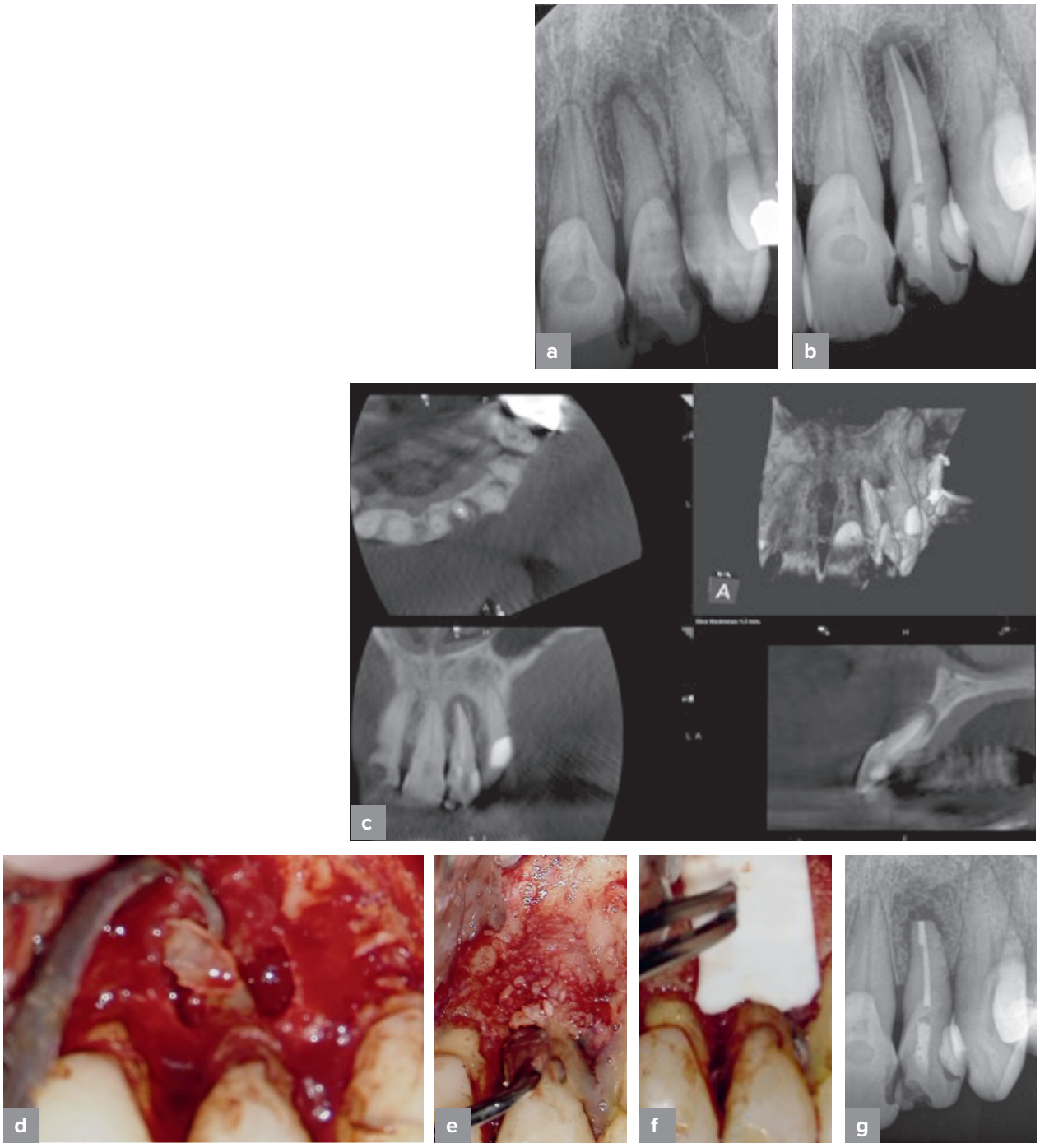


▲ **FIG 12-9** Cemental tear mimicking persistent apical periodontitis in the maxillary left lateral incisor. *(a)* Preoperative radiograph. The apical cemental tear went unnoticed, so endodontic treatment was performed. *(b)* Radiograph from the 36-month posttreatment evaluation. The cemental tear was now recognized. *(c)* CBCT image of the apical cemental tear. *(d to f)* Clinical photographs of cemental tear removal and guided tissue regeneration with Emdogain, bone graft material, and a collagen membrane. *(g)* Periapical radiograph from the 6-month post-treatment evaluation.

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## Poor endodontic treatment

The importance of quality endodontic treatment cannot be overstated. The causes of apical periodontitis and acute or chronic apical abscesses that persist after poor-quality endodontic treatment are similar to, if not the same as, those associated with the initial lesion. Poor-quality initial treatment typically fails to eliminate the causative agents generally comprising live pathogens such as bacteria, fungi, and viruses. Poor-quality initial treatment may also introduce extrinsic factors such as extruded dental materials, dentin, and cementum fragments, as well as remnants of paper points or cotton pellets. The clinician should carefully evaluate which of the causes of persistent disease are present and choose nonsurgical retreatment or surgery to offer the best chances for healing (Rotstein and Simon). CBCT is useful in identifying apical periodontitis and anatomy such as additional roots that may not be evident with periapical radiographs alone (Abella et al). When evaluating endodontically treated molars and premolars, Karabucak et al found that approximately 23% of these previously treated teeth had untreated canals; the maxillary molars had the highest incidence. Furthermore, previously untreated canals were 4.38 times more likely to have apical periodontitis.

The prognosis of quality endodontic retreatment is typically favorable, particularly if technical improvements can be made. In a retrospective study of 2,000 cases performed by a specialist with 18- to 24-month posttreatment evaluation, Imura et al found the success rate of initial endodontic treatment to be 94%, while that of retreatment was 85.9%, with preoperative periapical radiolucency being a strong predictor of lower success rates. Farzaneh et al found the overall healed rate of orthograde endodontic retreatment to be 81%, with 93% of the teeth studied being asymptomatic and functional at both the 4-year and 6-year posttreatment evaluation appointments. Inadequate initial root canal treatment was associated with higher success of endodontic retreatment; teeth with adequate prior treatment had an approximately seven-times higher chance of nonhealing after retreatment, suggesting factors such as foreign body reaction, presence of a true cyst, and extraradicular infection as possible reasons for failure that could be addressed surgically.

Other situations may make orthograde retreatment not feasible or successful and apical surgery more appropriate, with a higher chance of success. Examples include restorations that may be difficult or cost-prohibitive to deconstruct; separated instruments that may be impossible to remove due to their size or position in the root; difficult apical anatomy with ramifications, lateral canals, and isthmuses; and procedural errors such as ledges or apical transportation (Kim and Kratchman).

## Poor restoration

Restoration quality can affect both the success of endodontic treatment and the periodontal health of the teeth. Restorations with improper contours, open interproximal contacts, or margins that violate the biologic width all negatively affect the periodontal health. Ill-fitting restorations, improper use of restorative materials, and recurrent caries can all contribute to coronal microleakage, and the resulting contamination of the root canal system can lead to endodontic failure. In a laboratory setting, it has been shown that canals obturated with gutta-percha and sealer with either lateral compaction or warm vertical compaction will have bacterial contamination at all levels within 30 days when the coronal gutta-percha is exposed to human saliva (Khayat et al). A 1- to 2-mm-thick glass-ionomer orifice barrier placed over the canals and pulpal floor is effective in minimizing coronal microleakage after endodontic treatment and is typically a worthwhile adjunctive procedure (Maloney et al).

Controversy exists as to whether the quality of restoration or the quality of endodontic treatment plays a more significant role in treatment failure. Ray and Trope suggest that the quality of the restoration is more important and found that the odds of teeth not having apical periodontitis was 11.32 times higher when good restorations were present and 4.32 times higher when the quality of endodontic treatment was adequate. When both the restoration and endodontic treatment were poor, the success rate was approximately 18%, and when both the restoration and endodontic treatment were adequate, the success rate was near 82%. In contrast, when evaluating the influence of coronal restorations on the presence of apical periodontitis in 1,001 endodontically treated teeth, Tronstad et al found that the quality of the endodontic treatment was the most important factor associated with periapical health; however, if the quality of endodontic treatment was good, a high-quality definitive restoration improved the endodontic success rate by approximately 10%, showing that a quality coronal seal provided by the restoration is also important. A review of the literature confirms that the combination of high-quality endodontic and restorative treatment gives the best chance for healing and minimizes the incidence of apical and periodontal complications (Gillen et al).

When looking at tooth survival, the quality and presence of a definitive restoration is paramount. In an epidemiologic study involving over 1 million patients who completed initial root canal treatment, Salehrabi and Rotstein found that 97% of teeth survived at least 8 years; of the teeth requiring extraction, 85% did not have a full-coverage restoration placed. More recently, Pratt et al investigated the survival rates of


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endodontically treated teeth and reported survival rates of 84% for teeth receiving crowns, 71% for teeth receiving core buildups, and 58% for teeth that were not permanently restored; importantly, teeth receiving crowns over 4 months after endodontic treatment were three times more likely to be extracted than those receiving a full-coverage restoration prior to 4 months, and 60% of the extractions stemmed from crown fractures. Both studies illustrate the importance of the coronal restoration in the prevention of fracture and coronal leakage.

## Perforation

Root perforation, whether it be iatrogenic, resorptive, or caries-related, creates a communication between the root canal space and the periodontium. This communication creates unique treatment challenges, which may include internal or external surgical repair, orthodontic extrusion, forced eruption, extraction and repair with intentional replantation, or extraction with prosthetic replacement or space acceptance. Regarding iatrogenic perforation, Seltzer et al (1970) studied intentional perforations in rhesus monkeys and found that damage to the periodontium occurs universally, with the inflammatory reaction ranging from mild to severe; in addition, chronic inflammation was always noted. The prognosis for perforation treatment is dependent on the time from diagnosis to treatment and the size and location of the perforation (Fuss and Trope). Perforations treated at the time of perforation (or shortly after), small perforations, and those coronal and apical to but not at the alveolar crest have favorable prognoses for treatment as long as aseptic conditions are maintained during the repair procedure; on the other hand, large perforations and those with delayed treatment (likely with bacterial contamination) have questionable prognoses (Fuss and Trope).

The introduction of mineral trioxide aggregate (MTA) in 1993 marked a significant improvement in the materials available for perforation repair. Lee et al (1993) found MTA to be significantly superior to IRM and amalgam in terms of less leakage and less tendency for overfilling in vitro. In 1998, MTA became commercially available as ProRoot MTA (Dentsply Sirona) and subsequently has proven to be a reliable repair material. With long-term follow-up of at least 1 year, MTA has been shown to reliably lead to normal healing of the periodontium following perforation repair regardless of whether there were radiolucent lesions at the perforation sites at the time of repair (Main et al). Today, MTA and other calcium silicate–based materials, collectively known as *bioceramics*, are commonly used in endodontic practice to repair root perforations. Multiple studies have shown the long-term success rate of perforation repair to be quite good. Krupp et al found that 73.3% of cases healed after 1 to 10 years of posttreatment evaluation; the presence of a preoperative lesion and contact with the oral environment were correlated with lower success rates. Mente et al reported that 86% of cases healed over 12 to 107 months, with more experienced providers tending to have greater success. In a systematic review and meta-analysis, Siew et al found a pooled success rate of 72.5% for all repair materials compared to 81% with MTA, and the presence of a preoperative radiolucent area adjacent to the perforation in the mandibular arch was the only factor to significantly reduce the success of treatment.

The treatment of perforations stemming from internal inflammatory resorption is similar to that for iatrogenic origin, bearing in mind that bacterial contamination will likely be present, which makes this treatment similar to delayed repair. The treatment of external invasive resorptive perforations differs considerably from that of iatrogenic perforations, having the additional goals of mechanically cleaning the resorptive defect and destroying the resorbing tissue with the application of trichloroacetic acid followed by restoration, and may involve both internal and external surgical repair; success rates drop as the apical extension and circumferential spread increases (Heithersay).

Regardless of the type of perforation, advances in techniques and materials with timely, aseptic repair have made the treatment predictable, with known prognostic factors that can be discussed with patients to help them make informed decisions regarding treatment. Figure 12-10 shows a large iatrogenic perforation that went unrepaired for years with significant periodontal bone loss and an unfavorable prognosis. Figure 12-11 demonstrates an iatrogenic perforation treated with extraction, repair, and intentional replantation. Figure 12-12 illustrates examples of external invasive cervical root resorptions with and without periodontal attachment loss, both treated by internal repair with a bioceramic (Biodentine, Septodont).

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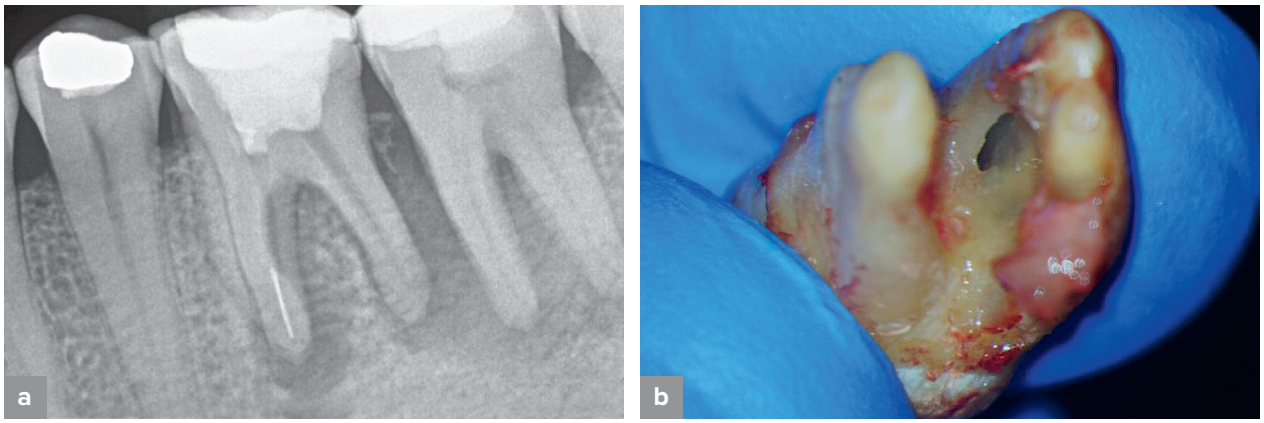
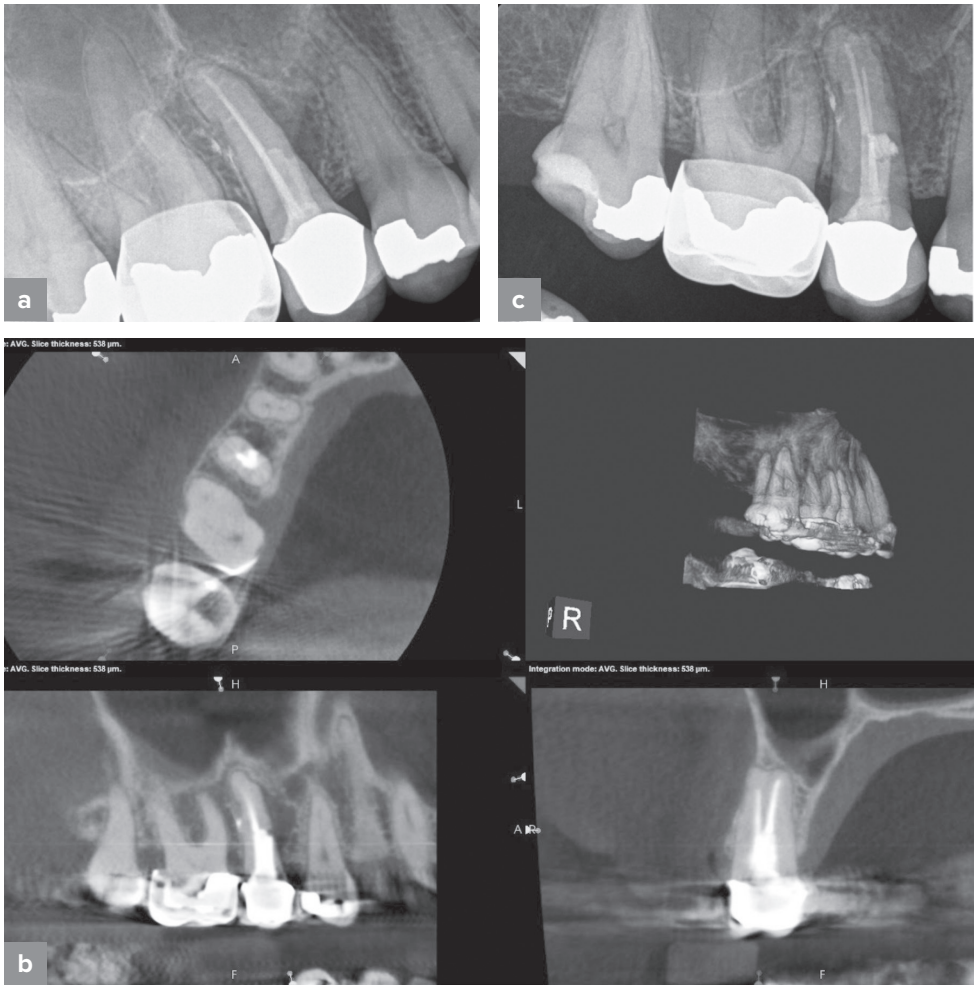



▲ **FIG 12-10** Iatrogenic perforation in the mandibular left first molar. *(a)* Periapical radiograph showing a large perforation with bone loss from the apex to the furcation. *(b)* Clinical photograph of the perforation after extraction.




◀ **FIG 12-11** Iatrogenic perforation in the maxillary right second premolar. *(a)* Periapical radiograph showing iatrogenic perforation from off-angle post placement. *(b)* CBCT image showing periodontal bone loss centered on the perforation. *(c)* Immediate postoperative radiograph after extraction, repair with a bioceramic putty, and intentional replantation.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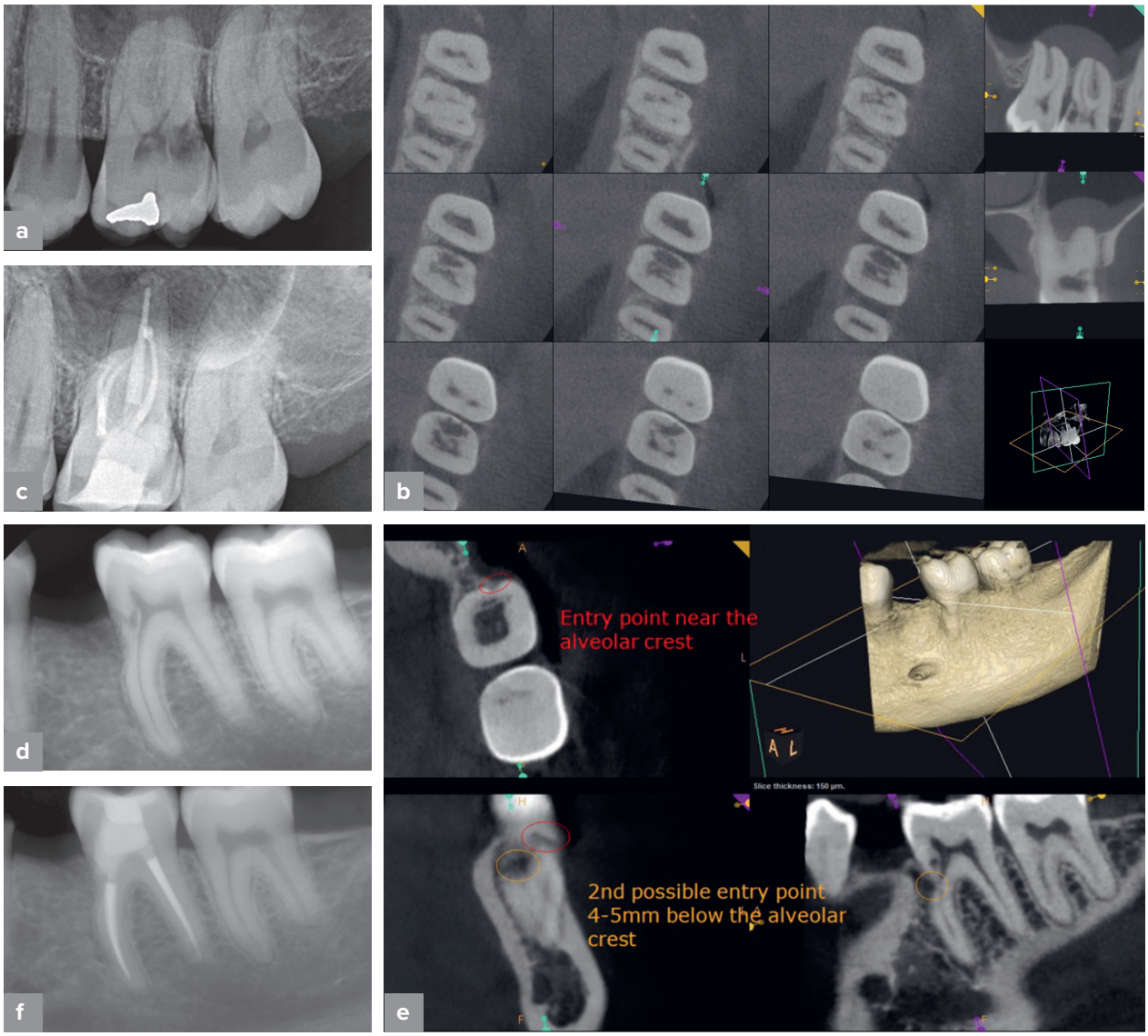


▲ **FIG 12-12** Resorptive perforations in a maxillary left first molar *(a to c)* and mandibular left first molar *(d to f)*. *(a and b)* Periapical and CBCT images showing external invasive cervical root resorption entering at the distobuccal aspect of the maxillary left first molar with periodontal bone loss at the area of the resorptive perforation. The pulp tested necrotic. *(c)* Immediate postoperative radiograph after root canal treatment and internal repair of the resorptive perforation. *(d and e)* Periapical and CBCT images showing mesial external invasive cervical root resorption with no periodontal bone loss in the mandibular left first molar. In this case, the tooth tested as having normal pulpal and apical tissues. *(f)* Immediate postoperative radiograph after root canal treatment and internal repair of the resorptive perforation.

## Trauma

The pulp, periodontal ligament, and supporting alveolar bone may be involved in traumatic dental injuries, classified as follows:

- Enamel infraction
- Uncomplicated crown fracture of enamel only
- Uncomplicated crown fracture involving dentin
- Complicated crown fracture
- Uncomplicated crown-root fracture
- Complicated crown-root fracture
- Root fracture
- Alveolar fracture
- Concussion
- Subluxation
- Extrusive luxation
- Lateral luxation
- Intrusive luxation
- Avulsion

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Injuries such as enamel infraction and concussion do not result in loss of tooth structure but may impact the health of the dental pulp over time. Injuries of greater magnitude can injure the enamel, dentin, pulp, periodontal ligament, and/or alveolar bone. The most recent comprehensive reviews from the International Association of Dental Traumatology provide an extensive summary of these injuries for permanent and primary teeth, including guidelines for examination, treatment, follow-up, and outcomes (Bourguinon et al, Fouad et al, Day et al, Levin et al).

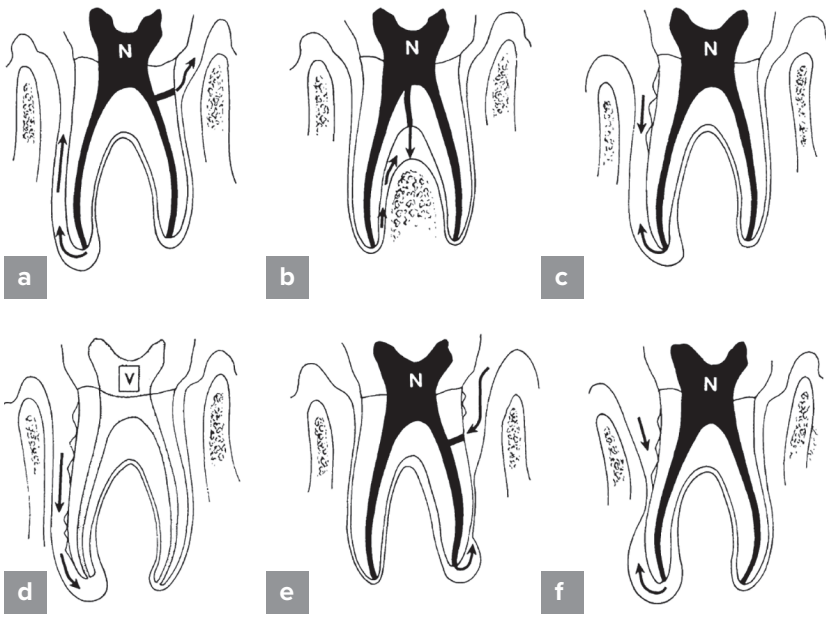
## Differential Diagnosis of Endodontic-Periodontal Lesions

The diagnosis of purely pulpal or periapical pathosis or periodontal disease is relatively straightforward. However, when there is more than one clinical and radiographic finding, the differential diagnosis becomes increasingly complex. With a single disease entity, the diagnosis, treatment plan, and therapy are generally clear-cut. When both endodontic and periodontal pathoses present in a patient, the diagnosis, treatment plan, and therapy must be systematic and managed correctly. The clinician must rely on multiple diagnostic procedures including a complete medical and dental history. A thorough clinical examination should include an extraoral and intraoral examination, periodontal probing, mobility testing, and sensibility/pulp testing. Radiographic interpretation of periapical and bitewing images and advanced radiographic techniques such as CBCT are essential. Radiographs tracing sinus tracts with gutta-percha are also required. Once all the potential problems are identified, a differential diagnosis is rendered, and a final diagnosis can be made.

When there are both endodontic and periodontal diseases present, or potentially present, it is important to determine the possible pathways for the development of the lesions. Understanding the pathogenesis of the disease(s) will aid the clinician in determining treatment, the order of treatment, and the possible prognosis. The classification of endodontic-periodontal disease leads to an understanding of the etiology of lesions and their relationships. This determines the order of which treatment should come first and which should follow.

## Classification of Endodontic-Periodontal Lesions

There have been several classifications of endodontic-periodontal lesions, and they differ slightly among the various authors (Amen, Begin, Oliet and Pollock, Simon and Jacobs). One of the more widely accepted classifications is the one proposed by Simon et al, which divides endodontic-periodontal lesions into five categories: *(1)* primary endodontic lesions, *(2)* primary endodontic lesions with secondary periodontal involvement, *(3)* primary periodontal lesions, *(4)* primary periodontal lesions with secondary endodontic involvement, and *(5)* true combined lesions (Fig 12-13).




◀ **FIG 12-13** Classification of endodontic-periodontal lesions. *(a and b)* Endodontic lesion. The pathway of fistulation is evident through the periodontal ligament from the apex or a lateral canal *(a)*. Fistulation through the apex or a lateral canal may cause bifurcation involvement *(b)*. *(c)* Primary endodontic lesion with secondary periodontal involvement. The existing pathway resembles that of endodontic lesions (see part *a*), but with the passage of time, periodontitis with calculus formation begins at the cervical area. *(d)* Periodontal lesion. This is the progression of periodontitis to apical involvement. Note the vital pulp. *(e)* Primary periodontal lesion with secondary endodontic involvement. The primary periodontal involvement at the cervical margin and the resultant pulpal necrosis once the lateral canal is exposed to the oral environment result in this clinical situation. *(f)* True combined lesion. The two separate lesions are heading to a coalescence, which will form the "true" combined lesion. (Reprinted with permission from Simon et al.)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## Primary endodontic lesions

Primary endodontic lesions appear clinically in teeth with necrotic pulps, which leads to apical periodontitis. A sinus tract develops from the apical lesion, which drains from the apical lesion via the periodontal ligament to the gingival sulcus. The sinus tract can often be mistaken for a periodontal pocket, which would also have a deep probing depth. A primary endodontic lesion can also develop if the bacterial byproducts from the necrotic pulp drain through a lateral canal; in this case, a sinus tract develops that drains through the periodontal ligament and through the gingival sulcus, which results in a periodontal pocket. Additionally, there could be drainage from the bacterial byproducts and inflammatory mediators of the necrotic pulp through the apical foramen or furcation canals into the furcation area, which would give the appearance of a periodontal lesion in the furcation. Pulp sensibility tests to determine pulpal necrosis are an important diagnostic step, as is tracing the sinus tract with a gutta-percha point to determine the origin of the drainage. The radiographic appearance may be deceiving and resemble a periodontal defect. Unless the sinus tract has been present for an extended period of time, probing may not detect plaque or calculus in the sinus tract. The primary endodontic lesion is not a periodontal defect but is in fact a draining sinus tract from an endodontic lesion. A primary endodontic lesion will heal with endodontic therapy alone.

## Primary endodontic lesions with secondary periodontal involvement

In the presence of a long-standing unresolved primary endodontic lesion, the sinus tract that is draining through the gingival sulcus will accumulate deposits of plaque and calculus. This leads to periodontal inflammation and breakdown secondary to the endodontic lesion. Periodontal probing will show plaque and calculus deposits as well as signs of periodontal inflammation, including bleeding on probing and suppuration. Besides the findings from periodontal probing, there will be a necrotic pulp. Treatment for the primary endodontic lesion with secondary periodontal involvement includes endodontic therapy followed by periodontal therapy. The prognosis depends on healing of both the endodontic lesion and the periodontal defect. The bone loss that is a result of the endodontic pathosis should heal with adequate endodontic treatment. The bone loss due to the periodontal component should heal if it can be effectively treated; however, bone loss due to periodontal destruction may not heal to its original position.

## Primary periodontal lesions

Primary periodontal lesions are the result of chronic periodontitis. Clinical findings include deep periodontal probing depths, plaque and calculus accumulations, and gingival and periodontal inflammation with bleeding and suppuration upon probing. Probing of these defects reveals a wide periodontal defect, as opposed to a primary endodontic lesion where the probing is into a very narrow pocket. There is bone loss indicative of periodontal disease. The dental pulp responds to pulp sensibility testing, indicating a vital pulp in most cases. Treatment involves periodontal therapy alone without endodontic treatment, and prognosis depends on the success of periodontal therapy.

## Primary periodontal lesions with secondary endodontic involvement

As periodontal disease progresses apically, the bone loss may expose lateral canals or the apical foramen to bacteria from the oral environment. This can lead to pulpal necrosis secondary to the primary periodontal disease. The radiographic appearance is the same as seen in primary endodontic lesions with secondary periodontal involvement. If these teeth are treated with periodontal procedures, they may not heal because of the endodontic component caused from pulpal necrosis. Again, pulp sensibility tests should be completed to determine pulpal vitality or necrosis. Treatment of primary periodontal lesions with secondary endodontic involvement requires endodontic therapy followed by periodontal therapy.

## True combined lesions

The final class of endodontic-periodontal lesions listed by Simon et al are true combined lesions. A tooth with a periapical lesion that developed as a sequela of a necrotic pulp may, at the same time, have a periodontal lesion as a result of periodontitis. Each of these lesions is formed independently and can continue to progress individually until the two merge. The clinical and radiographic presentation is one of two separate lesions that then combine to form a larger lesion where the former separate entities are now indistinguishable from each other. A common condition classified as a true combined lesion is the vertical root fracture, where there is a deep periodontal pocket and a periapical lesion.

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**TABLE 12-1** Endodontic-periodontal lesion classification from the 2017 World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions

| | |
|---|---|
| **Endodontic-periodontal lesion *with* root damage** | 1. Root fracture or cracking<br>2. Root canal or pulp chamber perforation<br>3. External root resorption |
| **Endodontic-periodontal lesion *without* root damage** | *Grade 1:* Narrow, deep periodontal pocket in one tooth surface<br>*Grade 2:* Wide, deep periodontal pocket in one tooth surface<br>*Grade 3:* Deep periodontal pockets in more than one tooth surface |

## Concomitant pulpal and periodontal lesions

Belk and Gutmann added an additional category to the classification of endodontic-periodontal lesions. They termed the new category *concomitant pulpal and periodontal lesions*. In this category of lesion, there are two distinct lesions, each with its own etiology and each independent of the other; they are not combined but are separate and exist concomitantly. Each of these lesions should be treated separately, starting with the endodontic condition followed by the periodontal condition.

## 2017 World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions

The 2017 World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions outlined a classification for endodontic-periodontal lesions (Papapanou et al). They listed two broad classifications: *(1)* endodontic-periodontal lesions with root damage and *(2)* endodontic-periodontal lesions without root damage (Table 12-1).

# Lateral Periodontal Cyst

The lateral periodontal cyst is mentioned here because, radiographically, it can resemble a lesion arising from a necrotic pulp and can cause loss of bone along a lateral surface of a root via an accessory canal, allowing release of inflammatory mediators and bacterial byproducts. It can also be mistaken for a cyst that occurs from periodontal inflammation. However, endodontic and periodontal therapy are NOT indicated in these lesions. The treatment for lateral periodontal cysts is enucleation, with care not to damage the roots that are adjacent to the lesion.

According to Neville et al and Altini and Shear, the lateral periodontal cyst is a relatively rare developmental odontogenic cyst. It makes up less than 2% of all epithelial-lined cysts of the jaws and usually occurs along the lateral root surfaces of teeth with vital pulps. Lateral periodontal cysts are typically less than 1.0 cm in diameter and are usually seen in the fifth to seventh decades of life. They are seldom observed in patients younger than 30 years old. Lateral periodontal cysts are found mostly in the mandibular premolar, canine, and lateral incisor region (75% to 80%). Their other location is the maxillary premolar, canine, and lateral incisor region (19%). Lateral periodontal cysts are asymptomatic and are discovered on radiographic examination. The radiographic findings are not diagnostic, as they cannot be distinguished from odontogenic keratocysts that develop between roots or pulpal or periodontal inflammatory lesions.

Lateral periodontal cysts are thought to form from the dental lamina. Histologically, the lateral periodontal cyst has a noninflamed thin fibrous wall. The epithelial lining is only 1 to 3 cells in width, with flattened squamous cells and a few cuboidal cells. Clear glycogen-rich cells are seen within the epithelial lining and in the fibrous wall.

The lateral periodontal cyst can present as a polycystic lesion, and this finding is termed a *botryoid odontogenic cyst*. The botryoid odontogenic cyst can appear radiographically as either a multilocular lesion or a unilocular lesion.


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## Prognosis of Endodontic-Periodontal Lesions

The prognosis after treatment of endodontic-periodontal lesions depends mainly on the specific diagnostic classification. The results of pulp sensibility testing and the extent of the periodontal defect are key factors in predicting prognosis. Primary endodontic disease and primary periodontal defects are more easily treated than combined lesions, and better outcomes should be expected. Primary endodontic disease presents with an infected pulp that is necrotic. In primary periodontal lesions, the pulp is vital. Primary endodontic lesions with secondary periodontal involvement, primary periodontal lesions with secondary endodontic involvement, and true combined lesions are indistinguishable in appearance on radiographs.

Primary endodontic lesions should be treated with only endodontic therapy and should have a very favorable prognosis. Primary periodontal disease should only be treated with periodontal therapy, and the prognosis depends on how severe the periodontal disease is and the patient's healing response and maintenance.

Primary endodontic lesions with secondary periodontal involvement should first be treated endodontically. The patient should be evaluated 2 to 3 months after the endodontic therapy is completed, and any required periodontal treatment should be initiated after this evaluation. This evaluation period allows the clinician to observe what portion of the defect was due to endodontic disease and what extent the periodontal defect had upon the amount of bone loss (Chapple and Lumley).

Primary periodontal lesions with secondary endodontic involvement and true combined endodontic-periodontal lesions must be treated by both endodontic and periodontal therapy. The endodontic therapy should be completed first, because infected pulps can have a negative effect on periodontal healing. Blomlöf et al found that endodontic infections or treatment can impact marginal hard and soft tissue repair and healing after periodontal therapy. Patients with infected pulps who are prone to periodontal disease tend to show an increase in their periodontal disease (Jansson et al 1995a).

The outcome for primary periodontal defects with secondary endodontic involvement and true combined lesions depends principally on the severity of the periodontal disease, the patient's response, and periodontal maintenance. True combined endodontic-periodontal lesions generally have a more questionable prognosis. If endodontic therapy is adequate, the portion of the lesion from an endodontic origin will heal. Therefore, the prognosis of a combined lesion depends on the severity of the periodontal defect, the success of the periodontal therapy, the patient's response, and adequate periodontal maintenance.

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# 13

# ROOT RESORPTION

Linda Levin

Root resorption is a rare occurrence in permanent teeth. Unlike bone, which undergoes resorption and apposition as part of a continual remodeling process, the roots of permanent teeth are not normally resorbed (Hammarström and Lindskog 1985). Only resorption of primary teeth during exfoliation is considered physiologic (Harokopakis-Hajishengallis, Yildirim et al).

## Mechanisms of Resorption

Even under conditions that would normally result in bone resorption, such as alterations in oxygen tension, hormonal fluctuations, locally produced chemical mediators, or electrical currents, the root is resistant to resorption on both its external and internal surfaces. Although several hypotheses have been proposed, the exact mechanism by which the resorptive process is inhibited is unknown. One hypothesis maintains that the remnants of Hertwig's epithelial root sheath (HERS) surround the root like a net and impart resistance to resorption and subsequent ankylosis (Orban, Loe and Waerhaug, DiAngelis et al, Zaidi et al, Xiong et al) (Fig 13-1). This theory is supported by evidence that the cells of HERS are involved in cemental repair subsequent to resorption by sequential elaboration of specific matrix proteins, namely osteopontin, bone morphogenetic protein 2, and ameloblastin (Hasegawa et al). In addition, studies using an immortalized HERS-derived cell line suggest that cells from this root sheath can produce a mineralized extracellular matrix that resembles acellular cementum (Zeichner-David et al).

A second hypothesis is based on the premise that the covering of cementum and predentin on dentin is essential for the resistance of the tooth root to resorption (Fig 13-2). There is support for this hypothesis in that it has long been noted that osteoclasts will not adhere to unmineralized matrix (Nakamura et al 1996). However, it has been shown that integrins on the osteoclast surface receive regulatory signals directly from bone mineral, which results in changes in the cytoskeleton that promote activation, binding, and formation of a sealing zone (Liu et al, Saltel et al 2004, Takahashi et al, Saltel et al 2008). Osteoclasts bind

▲ **FIG 13-1** Hertwig's epithelial root sheath is formed from the apical extent of the enamel organ and may impart resistance to resorption. D = dentin; O = odontoblasts; P = pulp.

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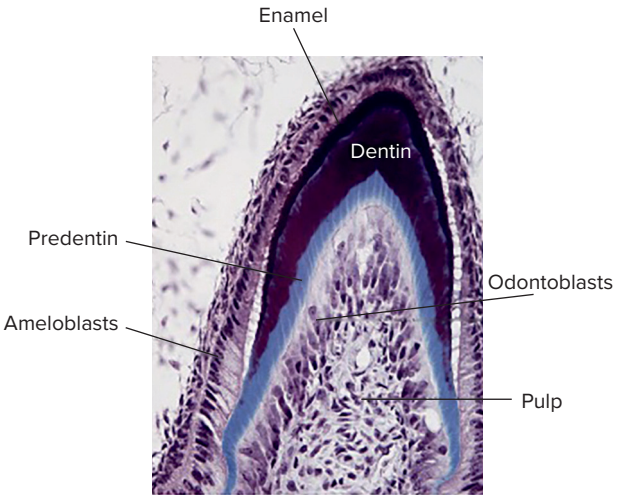
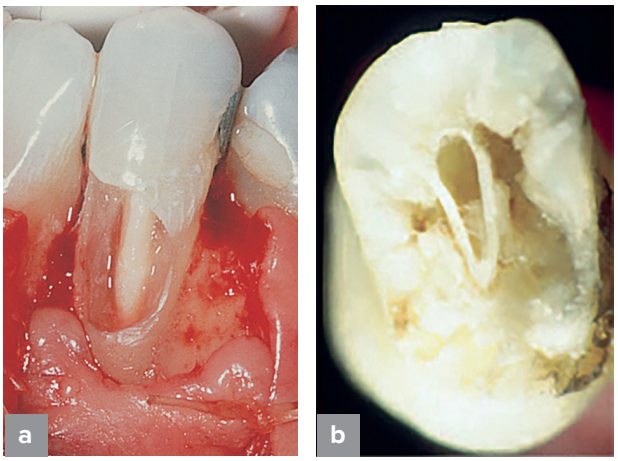



▲ **FIG 13-2** Predentin (stained blue) covers the more mineralized dentin surface and likely provides protection from resorption.




▲ **FIG 13-3** *(a and b)* Extensive invasive cervical resorption into dentin, but the pulp canal is not penetrated, most likely due to substances like osteoprotegerin that inhibit the resorptive process.

to extracellular proteins containing the arginine-glycine–aspartic acid (RGD) sequence of amino acids via specific membrane receptors known as *integrins* (Duong et al). Integrins are a family of cell-surface adhesion glycoproteins containing different α and β subunits. It is generally accepted that the $\alpha_V\beta_3$ integrin plays a central role in osteoclast migration, polarization, and adhesion (Nakamura et al 2007, Faccio et al). Extracellular proteins containing the RGD peptide sequence are bound to calcium salt crystals on mineralized surfaces and serve as osteoclast binding sites. In its normal state, the most external aspect of cementum is covered by a layer of cementoblasts over a zone of unmineralized cementoid and therefore does not present a satisfactory surface for osteoclast binding. Internally, the dentin is covered by predentin matrix, a similarly organic surface. This lack of RGD peptides in both cementum and predentin reduces osteoclast binding and confers resistance to resorption. Numerous studies lend support to this theory (Polson and Caton, Stenvik and Mjor, Wedenberg). It has also been demonstrated in cases of extensive external root resorption that the circumpulpal dentin, in close proximity to the predentin, is spared, even though most of the peripheral dentin has been resorbed (Fig 13-3). Conversely, damage to cementum or predentin increases the probability of osteoclast-induced resorption, which can occur in teeth damaged during avulsion or other traumatic injury. Localized replacement resorption and mild to severe external inflammatory root resorption with pulpal involvement have been demonstrated when the cementum was damaged by inadvertent contact of root surfaces with miniscrew implants employed for anchorage during orthodontic treatment (Kadioglu et al, Chen et al 2008, Hembree et al). Although this hypothesis has both experimental support and clinical implications, other hypotheses have also been advanced.

Another possible explanation for the relative resistance of permanent teeth to root resorption is that intrinsic factors found in predentin and cementum, such as amelogenin or osteoprotegerin (OPG), act as inhibitors of resorptive cells (Hatakeyama et al, Yagi et al 2009). OPG, a member of the tumor necrosis factor (TNF) superfamily, inhibits osteoclast-mediated bone loss. OPG acts as a decoy receptor by binding to the receptor activator of nuclear factor κB ligand (RANKL). Binding of OPG reduces RANKL concentration and thereby inhibits its ability to bind to receptor activator of nuclear factor κB (RANK) receptors on the surface of osteoclast precursors (circulating monocytes) and stimulate osteoclast production (osteoclastogenesis) (Kong et al, Khosla, Tyrovola et al, Boyce and Xing, Wright et al). Cementoblasts have been shown to constitutively express OPG in vitro (Nociti et al). Human gingival fibroblasts, human periodontal ligament (PDL) cells, human pulp cells, and dental pulp stem cells also produce OPG (Wada et al, Sakata et al, Uchiyama et al, Kuntz et al, Kanji et al). The role of OPG in the suppression of root resorption is supported in OPG knockout mice, where OPG-deficient mice developed root resorption (Liu et al).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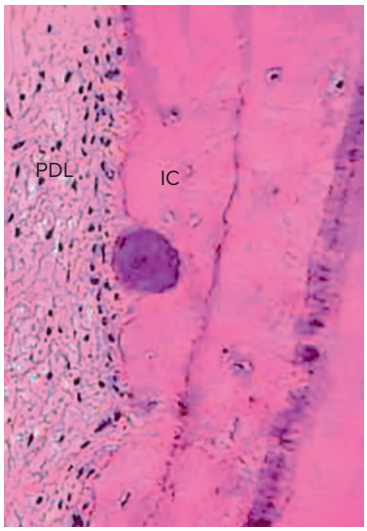


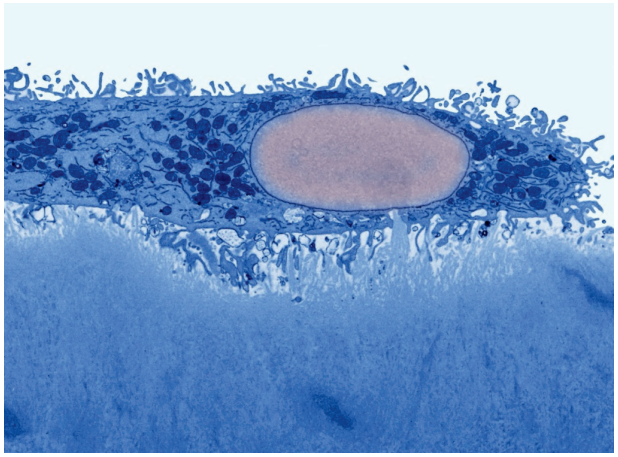

▶ **FIG 13-4** If the intermediate cementum (IC) is penetrated, any pulpal toxins that may be present will cause periodontal inflammation, with bone and root resorption (hematoxylin-eosin [h&e] stain; original magnification ×25). (Reprinted with permission from Consolaro et al.)

Finally, another hypothesis applicable to certain types of external root resorption involves the barrier formed by the less highly calcified intermediate cementum or the cementodentinal junction (Ho et al 2004, Ho et al 2005, Ho et al 2009) (Fig 13-4). The intermediate cementum, the innermost layer of cementum, creates a barrier between the dentinal tubules and the PDL (Saygin et al, Andreasen 1981a, Andreasen 1981b). Under normal circumstances, this barrier does not allow irritants such as bacterial byproducts to pass from an infected pulp space to stimulate an inflammatory response in the adjacent PDL. However, if the intermediate cementum is lost or damaged, then proinflammatory mediators may diffuse from an infected pulp space into the PDL, setting up an inflammatory response and subsequent external inflammatory root resorption. In addition, the collagen-rich barrier provided by intermediate cementum could prevent the attachment of osteoclasts to mineralized dentin even when external resorption has occurred in the peripheral, more highly mineralized cementum. When the intermediate cementum is lost or damaged, the osteoclasts may continue to induce resorption in the underlying dentin in the presence of inflammatory stimulators, resulting in the progression of external inflammatory root resorption to the radicular dentin. These different hypotheses are not mutually exclusive, and their relative contributions to various clinical conditions may also vary depending on the circumstances of each case.

## Biology of Resorption

It is now well established that a strong interplay among factors involved in hematopoiesis and immune function is responsible for osteoclastogenesis (Fischer and Haffner-Luntzer, Huang et al, Yahara et al) (Fig 13-5). In vivo studies have shown that embryonic macrophage precursors give rise to premacrophages and fetal monocytes that persist in adult tissue due to their ability to self-renew (Ginhoux and Guilliams). These embryonic precursors are necessary for normal bone development, tooth eruption, and development of the postnatal marrow space (Kurotaki et al). Hematopoietic stem cell precursors appear to contribute to the maintenance of osteoclast syncytia after birth, but both lines persist and can fuse to form multinucleated osteoclasts. The myeloid and B-cell transcription factor PU.1 is the earliest identifiable determinant of the osteoclast lineage (Tondravi et al, Kurotaki et al) and is actively involved in the regulation of $\beta_3$ integrin expression during osteoclast differentiation (Crotti et al 2008). Disruption of the *PU.1* gene in mice results in a deficiency in most macrophage groups and notably in osteoclasts (McKercher et al). Other factors involved include the lipopolysaccharide receptor CD14 and the early monocytic integrins CD11b and CD11c. CD11b is one of the recognized markers of osteoclast precursors in humans (Blair and Zaidi).

Once recruited, a variety of mediators such as hormones, integrins, transcription factors, and cytokines affect osteoclast differentiation, maturation, and function (Blair and Zaidi, Asagiri and Takayanagi, Shinohara and Takayanagi, Kim and Kim). The primary factors involved in pathologic root resorption are fewer, however, and are primarily or secondarily associated with the inflammatory response to infection. It is known that inflammatory mediators are potent stimulators of osteoclast and odontoclast recruitment and function. TNF; interleukins 1 (IL-1), 6 (IL-6), 11 (IL-11), 15 (IL-15), 17 (IL-17A, IL-17F), and 23 (IL-23r); transforming growth factor α (TGF-α); and prostaglandin $E_2$ influence stromal cells to produce pro-osteoclastogenic molecules. Receptor-ligand interactions perpetuate the process; these include RANK and RANKL, Ephrinβ2/



▲ **FIG 13-5** Transmission electron micrograph of an odontoclast on dentin. Note the complexity of the resorptive lacunae.

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EphB4, Fas/FasL, previously mentioned RGD-containing extracellular matrix molecules and $\alpha_V\beta_3$ integrin, as well as macrophage colony-stimulating factor (M-CSF) and cFms. Receptor-ligand binding initiates a cascade of signaling intermediates (Src, TNF receptor–associated factor 6, and PI3K), cytoplasmic phosphokinases (protein kinase B [Akt], c-Jun N-terminal kinases, p38, and extracellular signal–regulated kinase), transcription factors (c-Fos, c-Jun, NFκB, and nuclear factor of activated T cells, cytoplasmic 1), and effector molecules necessary for osteoclastic activity (matrix metalloproteinase 9 [MMP-9], vacuolar $H^+$-adenosine triphosphatase [V-ATPase], and cathepsin K) (Zaidi et al, Chen et al 2018).

Once bound to a substrate, osteoclastic activity involves removal of both the inorganic and organic components from the mineralized tissue (Sriarj et al). Dissolution of the mineralized component of bone and cementum by osteoclasts is largely dependent on acidification of the extracellular resorption lacunae by V-ATPases within the ruffled border membranes of the osteoclasts (Farina and Gagliardi, Xu et al, Kuno). After dissolution of the mineral phase, specific members of two specific classes of proteolytic enzymes—cysteine proteinases (cathepsin K) and matrix metalloproteinases (MMP-1, -2, and -9)—secreted by the osteoclasts contribute differentially to the degradation of the extracellular demineralized type 1 collagen matrix (Delaissé et al, Everts et al, Sundaram et al, Wilson et al).

Some of the mediators of inflammation-induced clastic function belong to the glycoprotein 130 (gp130) cytokine family that has been shown to play a key role in bone remodeling (Heymann and Rousselle). This family is a pleomorphic group that shares a common signal transducer (gp130) and includes cardiotrophin 1, oncostatin M, ciliary neurotrophic factor, IL-6, IL-11, and leukemia inhibitory factor. Of these factors, IL-6 and IL-11 appear to have the most profound effect on hard tissue resorption, and IL-6 has been shown to stimulate osteoclastogenesis in the presence of IL-1 but not in the presence of anti–IL-1 (Kurihara et al, Kespohl et al). This effect is dependent on the expression of the IL-6 receptor on osteoblastic cells and occurs in a dose-dependent manner (Udagawa et al). IL-6 also appears to reverse the inhibitory effects of extracellular $Ca^{2+}$ by reducing the ability of osteoclasts to detect extracellular $Ca^{2+}$ concentrations and is expressed by cells of the stromal/osteoblastic lineage (Sims 2016).

IL-11 is an osteoblast-derived mediator that induces prostaglandin synthesis and triggers differentiation of clastic cells. Cells of the osteoclast phenotype have been shown to express the IL-11 receptor gene, which in turn seems to be related sequentially to expression of the calcitonin receptor gene, a recognized osteoclast differentiation marker (Sims 2021). Furthermore, gp130 signal induction by IL-11 appears to be necessary for IL-1–induced osteoclast formation (Kespohl et al, Goldring et al, Heymann and Rousselle, Kwan Tat et al).

The singular defining feature of the mature osteoclast is function, namely the ability to resorb calcified tissues. Morphologically, the osteoclast is described as a multinucleated giant cell formed by the fusion of monocytic precursors (Asagiri and Takayanagi). This fusion is mediated by external stimuli such as M-CSF and RANKL (Cho et al, Kodama and Kaito). Multinucleation occurs late in osteoclastogenesis and appears to be regulated by master fusogens, dendritic cell–specific transmembrane protein (DC-STAMP), and osteocyte cell–specific transmembrane protein (OC-STAMP) (Yang et al, Yagi et al 2005). Odontoclasts differ slightly from their bone-resorbing counterparts in that they are usually smaller, have fewer nuclei, and have very small (if any) sealing zones (Lindskog et al 1983, Szewczyk et al). Nonetheless, these cells are thought to represent a variant of osteoclasts with morphologic differences that reflect the nature of the resorption substrate as well as the resorption kinetics of the cells (Asagiri and Takayanagi).

Dendritic cells, the mononuclear cells that initiate the adaptive immune response, share a common hematopoietic lineage with the multinucleated bone-resorbing osteoclasts and have been shown to have the ability to differentiate into osteoclast-like cells through a process regulated by innate and adaptive cytokines (Rivollier et al, Speziani et al, Mochizuki et al). Both human and murine dendritic cells have been shown to be osteoclast precursors that are able to differentiate into osteoclasts more efficiently than monocytes (Gallois et al). They also have the ability to influence osteoclastogenesis by the elaboration of DC-STAMP (Hartgers et al, Yagi et al 2005). As previously mentioned, this protein is considered an important regulator of osteoclast differentiation by acting as a major facilitator of cell fusion in osteoclast precursor cells (Li et al 2014, Courtial et al, Yagi et al 2005). Production of DC-STAMP is costimulated by RANK and immunoreceptor tyrosine-based activation motif–containing (ITAM-containing) receptors/adaptors (Li et al 2014). Because dendritic cells are present in the dental pulp, it is plausible that they can function as precursors/stimulators of clastic cells that are responsible for internal resorption of the canal space under stimulatory conditions.

On a cellular level, osteoclasts are usually large and have multiple nuclei. They possess a well-defined Golgi apparatus, numerous lysosomal vesicles, and high polarity, with the nuclei residing near the apical membrane and the vesicles in the ruffled border adjacent to bone (Teitelbaum, Novack and Teitelbaum). Characteristic morphologic features of the osteoclast are the ruffled border, formed by membrane and cytoplasmic undulations, and


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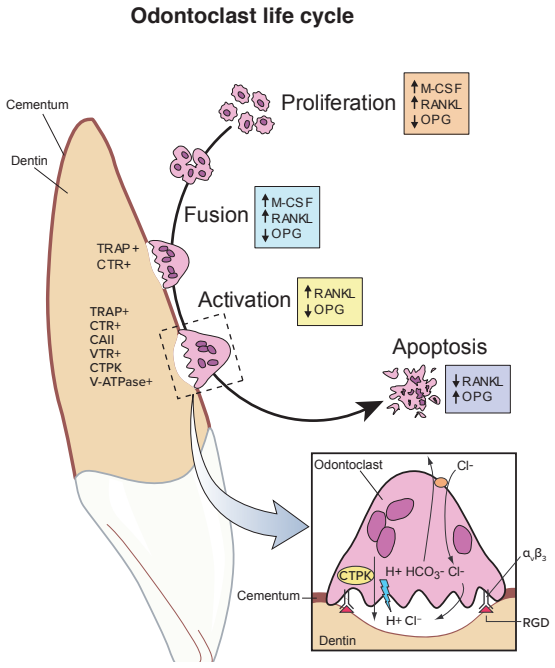
the sealing zone, consisting of the ventral surface of the osteoclast membrane in contact with the targeted bone or root surface forming the resorptive hemivacuole (Szewczyk et al). Scanning electron microscopy (SEM) has revealed that the ventral side of osteoclasts is complex and is composed of actin rings and podosomes that form the sealing zone (see Fig 13-5). Within the sealing zone there is a series of membrane folds that comprise the ruffled border (Szewczyk et al). The mature osteoclast is further defined by a series of molecular markers, each representing genes integral to clastic function (Del Fattore et al). These include transcripts coding for tartrate-resistant acid phosphatases, calcitonin receptors, vitronectin receptors, carbonic anhydrase II, cathepsin K, and V-ATPase (Aubin and Bonnelye).

The dynamic interactions between osteoclasts and the innate immune system have increasingly been recognized, resulting in a rapidly developing field of research known as *osteoimmunology* (Boyce et al, Wu et al, Nakashima and Takayanagi 2008, Lorenzo et al, Nakashima and Takayanagi 2009, Caetano-Lopes et al, Crotti et al 2015). There is increasing evidence that osteoclast precursors and osteoclasts modulate the differentiation of osteoblastic cells, regulate hematopoietic stem cell movement from the bone marrow to the bloodstream, and function as secretory cells that participate in immune responses. Osteoclasts also function as highly regulated innate immune cells of the bone that respond to stress and inflammatory changes in their microenvironment. Their interactions with the immune system are mediated not only by the release of cytokines and chemokines but also by direct cell-to-cell contact (Vig and Fernandes). This cross-talk is mediated by both cell surface–bound proteins as well as secreted proteins. Exosomes are extracellular vesicles that are secreted into the extracellular matrix to effect cell-to-cell signaling. They contain proteins, nucleic acids, and lipids and have been shown to play a crucial role in bone homeostasis (Vig and Fernandes, Kim et al).

An understanding of the complex interactions between osteoclasts and the immune system has provided a scientific basis for therapeutic approaches to diseases related to both the bone and immune systems. In the context of root resorption, elucidating the interaction of osteoclasts with the immune system may improve the understanding of the pathogenesis and provide solutions to the treatment of patients with idiopathic multiple external cervical resorptive lesions (Coyle et al). A model for the process of root resorption can be depicted based on what is known about odontoclasts combined with data extrapolated from studies of osteoclasts (Fig 13-6). As previously mentioned, initiation of root resorption has two requirements: *(1)* removal of the protective layer (predentin internally and precementum externally) from the root and *(2)* the presence of a noxious stimulus that results in an inflammatory response adjacent to the damaged external root surface. Given these conditions, the first step in root resorption is binding of the odontoclast to its substrate.

Various RGD peptide–containing proteins (eg, osteopontin, osteocalcin, bone sialoprotein, fibronectin, and vitronectin) have been shown to be involved in osteoclast binding (Nagasaki et al). Most notably, osteopontin has been shown to play an important role in regulating osteoclast recruitment and activation by binding to the osteoclast integrin receptor $\alpha_V\beta_3$ (Faccio et al, Nakamura et al 2007). Osteopontin deficiency resulted in osteoclast suppression and reduction in tooth resorption in a murine model (Chung et al). Osteopontin serves as a linker molecule so that one domain is bound to apatite crystallites in exposed dentin and another domain is bound to the integrin protein ($\alpha_V\beta_3$) extending from the osteoclast's plasma membrane (Karanth et al, Si et al, Icer and Gezmen-Karadag). Ameloblastin, another matrix adhesion protein, has also been shown to be osteoclastogenic (Lu et al). It is upregulated during orthodontic tooth movement and is produced by ameloblasts, cementoblasts, osteoblasts, as well as the epithelial rests of Malassez (Lu et al).

The binding of osteopontin to $\alpha_V\beta_3$ integrin facilitates clastic cell adhesion and subsequent establishment of the clear zone or extracellular resorbing compartment.




▲ **FIG 13-6** Odontoclast life cycle. Shaded boxes list molecules involved in osteoclast maturation, function, and demise. Tartrate-resistant acid phosphatase (TRAP), calcitonin receptors (CTRs), vitronectin receptors (VTRs), carbonic anhydrase II (CAII), cathepsin K (CTPK), and V-ATPase are molecular markers for the mature clastic phenotype.

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**TABLE 13-1 Molecular patterns that contribute to inflammation and osteoclastogenesis and their applicability to dental pathosis and odontoclast activation/function**

| Molecular pattern | Source | Mechanism | Dental etiology |
|---|---|---|---|
| Pathogen-associated molecular pattern (PAMP) or microbe-associated molecular pattern (MAMP) | Microorganisms (LPS, LTA, flagellin) | Infection | Infection due to caries, cracks |
| Damage-associated molecular pattern (DAMP) | Damaged host cells (tumor cells, dead or dying cells) | Ischemia/hypoxia, trauma | Trauma, orthodontics, impacted teeth |
| Xenobiotic-associated molecular pattern (XAMP) | Pollutants, chemicals, toxins | Environmental exposure | Dental materials, bleaching |

Osteopontin-$\alpha_V\beta_3$ interaction with odontoclasts stimulates a second messenger pathway (ie, gelsolin association with phosphatidylinositol-3-hydroxyl kinase via a $pp60^{src}$- and RhoA-dependent pathway) that mediates cytoskeletal rearrangement, ruffled border formation, polarization, and substrate adhesion (Giachelli and Steitz). Once the ruffled border has been formed, the odontoclast secretes an acidic solution containing proteolytic enzymes, specifically carbonic anhydrase and V-ATPase, into the extracellular resorbing compartment to effect decalcification (Teitelbaum). While the mechanisms of clastic cell adhesion are similar between bone and dentin substrates, it has been noted that clastic cells adhere 45% better to root dentin as compared to bone (Rumpler et al). Furthermore, in vitro studies have shown that there are comparatively more resorptive pits formed on dentin compared to bone (3,134 pits/$cm^2$ versus 449 pits/$cm^2$), indicating that dentin is a more osteoclastogenic substrate than bone (Geblinger et al).

In certain types of root resorption, bacteria play a significant role in the initiation and perpetuation of the resorptive process. Bacterially induced osteoclastogenesis is mediated by the interaction of specific molecular patterns presented by microorganisms, with pattern-recognition receptors (PRRs) expressed on host cells. The PRRs are toll-like receptors that represent a critical bridge between the adaptive and innate immune systems. PRRs recognize four types of molecular patterns: damage-associated molecular patterns (DAMPs), microbe-associated molecular patterns (MAMPs), pathogen-associated molecular patterns (PAMPs), and xenobiotic-associated molecular patterns (XAMPs) (Behzadi et al, Li and Chang). The binding of PRRs to these recognition patterns initiates both transcriptional and nontranscriptional responses of the innate immune system, resulting in the formation of inflammasomes. These are supramolecular protein complexes that are assembled in response to PRRs. Inflammasomes are responsible for stimulation, maturation, and secretion of proinflammatory cytokines as well as activation of inflammatory responses (Mbalaviele and Veis). It is likely that all four molecular patterns (DAMPs, MAMPs, PAMPs, and XAMPs) are involved in root resorption (Table 13-1). Bacteria and their byproducts can act through these recognition patterns to directly stimulate osteoclastogenesis. While lipopolysaccharide (LPS) and lipoteichoic acid (LTA) can stimulate fibroblasts, macrophages, osteoblasts, and other cells to produce osteoclastogenic cytokines and RANKL, they can also promote osteoclastogenesis by direct stimulation of osteoclast progenitors (Zou and Bar-Shavit).

During root resorption, organic matrix proteins and cytokines from the surrounding bone and dentin are released locally into the gingival crevicular fluid. The concentrations of dentin phosphophoryn and dentin sialoprotein were found to be higher in the gingival crevicular fluid of human subjects with severe root resorption. These two dentin-specific organic matrix proteins have been suggested as biologic markers for monitoring root resorption during orthodontic treatment (Balducci et al, Mandour et al). Osteopontin, OPG, and RANKL could also be detected in the gingival crevicular fluid derived from teeth with severe root resorption. An increased RANKL-OPG ratio was found to be correlated with an increase in bone resorption activity during orthodontic tooth movement (Wei et al).

## Role of the Dental Pulp in Resorption

The pulp plays an essential role in two types of resorption. The first type of resorption is external inflammatory root resorption. In external inflammatory root resorption, the necrotic infected pulp provides the stimulus for periodontal inflammation (Fouad). If the cementum has been


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damaged and the intermediate cementum is penetrated, as when the tooth sustains a traumatic injury, the inflammatory stimuli in the pulp space are able to diffuse through the dentinal tubules and induce an inflammatory response over large areas of the PDL. Without cemental protection, this will result in resorption of both tooth and bone.

The second type of resorption in which pulp tissue plays an important role is internal inflammatory root resorption. In internal inflammatory root resorption, the inflamed pulp is responsible for resorption of the root structure. The pathogenesis of internal root resorption is not completely understood. One theory maintains that coronal necrotic infected pulp provides a stimulus for inflammation in the more apical parts of the pulp. An alternative theory is based on the knowledge that apoptotic osteocytes induce the secretion of osteoclastogenic cytokines that enhance bone resorption (Gu et al, Kogianni and Noble). It is known that odontoblasts undergo apoptosis both during tooth development as well as in response to certain types of trauma (Franquin et al, Shigehara et al, Kitamura et al). In view of the parallels between dentin and bone physiology, it is probable that odontoblasts or pulp fibroblasts undergoing apoptosis in response to trauma induce cytokines that initiate and perpetuate the internal resorptive response. In rare cases in which the inflamed pulp is adjacent to a root surface that has lost its precemental protection, internal root resorption will result. Thus, both the necrotic infected pulp and the inflamed pulp can contribute to this type of root resorption.

## Classification of Root Resorption

Several authors have proposed classification systems for pathologic root resorption. All are based on etiology and/or location. Of those categories, there are multiple subcategories that have clinical significance in terms of both treatment and prognosis. All tooth resorption can be categorized as either external or internal. Further classification is then based on the specific tissues affected as well as the pathophysiology involved (Abbott and Lin, Ne et al, Heboyan et al). Box 13-1 shows one classification system proposed, listing 11 different types of tooth resorption (Abbott and Lin).

## External Root Resorption

Injury to the root surface can cause a loss of the protective cemental layer, providing a stimulus for inflammation. This type of resorption is self-limiting. The inflammatory mediators evoked by the mechanical damage stimulate the cellular inflammatory response. The character of the healing response is dependent on the extent of the initial damage and can therefore take several forms (Heboyan et al).

**BOX 13-1 A proposed classification system for root resorption**

**Internal resorption**
- Internal surface resorption
- Internal inflammatory resorption
- Internal replacement resorption

**External resorption**
- External surface resorption
- External inflammatory resorption
  - Apical
  - Lateral
- External replacement resorption
  - Transient
  - Progressive
- External invasive resorption
- External pressure resorption
  - Orthodontically induced inflammatory root resorption (OIIRR)

Adapted from Abbott and Lin.

### Localized injury: Transient apical breakdown

When the injury is localized (eg, after concussion or subluxation injury), mechanical damage to the cementum occurs, resulting in a local inflammatory response and a localized area of root resorption. This was first described by Andreasen and coined *transient apical breakdown* (Andreasen and Kahler, Andreasen 2003). This can occur subsequent to traumatic injuries such as luxation or concussion where the apical tissues are injured. If no further inflammatory stimulus is present, periodontal healing and root surface repair will occur (Fig 13-7). The resorption is localized to the area of mechanical damage, and treatment is not required because the resorption is free of symptoms and in most cases undetectable with routine radiography (Laux et al). However, in a minority of cases, small radiolucencies can be seen on the root surface if the radiograph is taken at a specific angle or if CBCT is used.

It is important not to misinterpret these cases as progressive. It is equally important to understand that the pulp is not involved in these cases. The stimulus is presumed to be a healing process to remove damaged apical tissue. When no potential inflammatory stimulus can be identified (ie, when a positive sensitivity test indicates a vital pulp), no endodontic treatment should be performed. Treatment is generally limited to clinical monitoring to verify that spontaneous healing is taking place.

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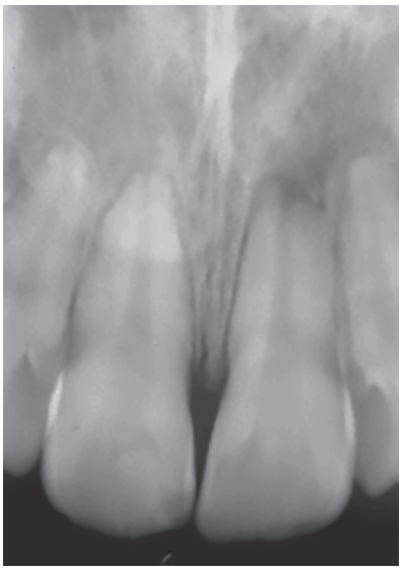
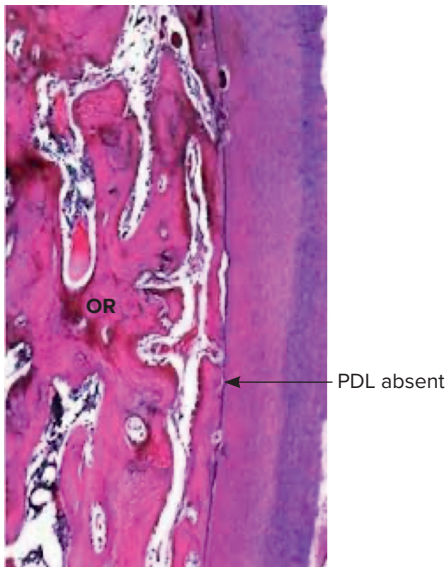
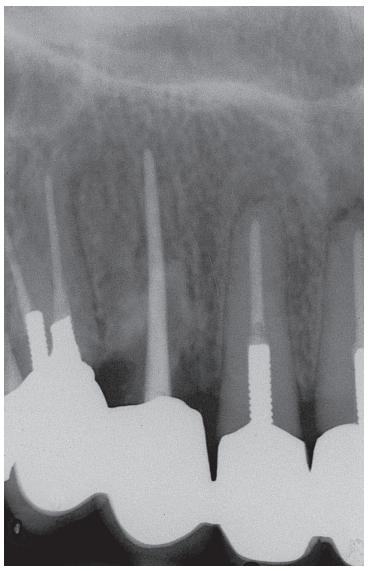


▲ **FIG 13-7** Transient external inflammatory root resorption. Note the lucent areas on the root surface. Because the pulp is vital, a wait-and-see strategy is observed. (Reprinted with permission from Cohenca et al 2003.)




▲ **FIG 13-8** Histologic appearance of active osseous replacement (OR). Without an intermediate PDL, bone attaches directly to the root. Areas of active root resorption are seen in the bone and root (h&e stain; original magnification ×100).



▲ **FIG 13-9** Radiographic appearance of osseous replacement. The bone that is replacing the root has a mottled appearance. Radiolucencies are not apparent in the adjacent bone.

## Diffuse injury: External or osseous replacement resorption

When the traumatic injury is severe (eg, intrusive luxation or avulsion with extended dry time) and involves diffuse damage on more than 20% of the root surface, an abnormal attachment may occur after healing takes place (Lindskog et al 1985). The initial reaction is an inflammatory response to the severe mechanical damage to the root surface. After the initial inflammatory response, a diffuse area of root surface will be devoid of cementum due to the injury. Cells in the vicinity of the denuded root compete to repopulate it, and often cells that are precursors of bone, rather than PDL cells, will migrate from the socket wall and populate the damaged root (Fig 13-8). The result is that bone comes in direct contact with the root, without the intermediate cementum to serve as an attachment apparatus. This phenomenon is termed *dentoalveolar ankylosis* (Hammarström et al, Krastl et al 2021) (Fig 13-9). In essence, dentin becomes part of the bone-remodeling process. Osteoclasts resorb root structure, and the resorptive lacunae are repopulated by osteoblasts, not odontoblasts or cementoblasts (Hammarström and Lindskog 1992). The osteoblasts then synthesize and deposit bone matrix directly on the denuded dentin, and mineralization ensues. The resultant hard tissue is bone, not dentin or cementum. This process can continue until the entire root is replaced by bone. While the injury that stimulates external replacement resorption (ERR) is often extreme, resulting in pulpal necrosis, it is possible that ERR can proceed with a vital pulp. It is also possible for ERR to stop with resumption of a normal PDL (Fig 13-10). When ERR occurs in children, eruption can be impaired, resulting in an alveolar deformity and infraocclusion of the tooth. Decoronation has been shown to facilitate resumption of proper alveolar development (Malmgren et al).

Treatment strategies for treating ERR (or osseous replacement resorption) are directed at avoiding or minimizing the initial inflammatory response. Steroids have been used as an intracanal medicament in animal studies evaluating healing after avulsion with long extraoral dry times (at least 60 minutes). These studies consistently showed better healing with steroids versus other medicaments (Sae-Lim et al, Bryson et al, Kum et al, Kirakozova et al). Growth factors, antibiotics, bisphosphonates, enamel matrix derivatives, and fluoride have all been investigated for the prevention of replacement resorption, with promising but variable success (Tuna et al). Five specific strategies should be considered: *(1)* Prevention of the initial injury should be emphasized by counseling or advocating use of mouthguards for athletic endeavors; *(2)* treatment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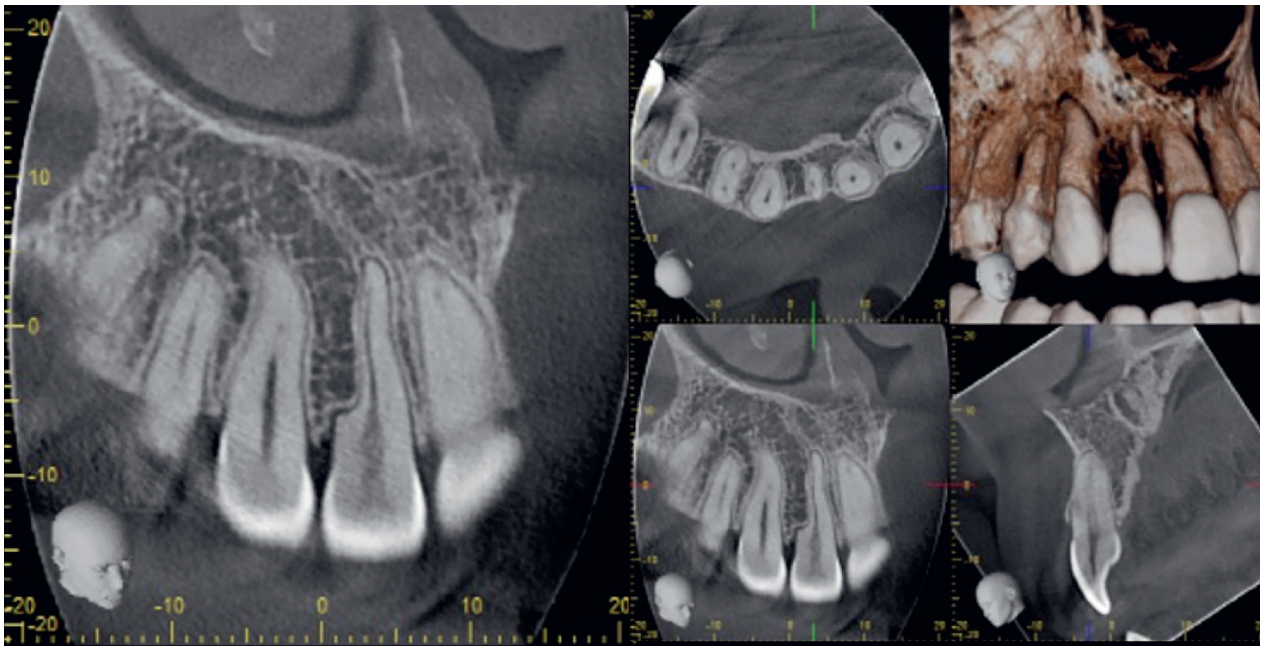

▲ **FIG 13-10** CBCT of healed replacement resorption. The maxillary left central incisor tested vital to cold and was asymptomatic. Because this patient is an adult, no treatment was recommended.

should minimize additional damage after the initial injury by gentle repositioning of luxated teeth or prompt replantation of avulsed teeth; *(3)* pharmacologic interventions that inhibit the initial inflammatory response should be considered; *(4)* the possibility of stimulating cemental (rather than osseous) healing should be considered; and *(5)* interventions that reduce the rate of osseous replacement, when inevitable, should be considered (DiAngelis et al, Fouad et al, Bourguignon et al).

## Inflammatory root resorption

There are four general types of inflammatory stimuli that cause external root resorption: *(1)* pressure-induced damage to the root surface, *(2)* microbial infection of the root canal system, *(3)* sulcular infection, and *(4)* chemical or mechanical damage secondary to bleaching or periodontal therapy (Heithersay et al, Patel and Pitt Ford, Zoya et al). Factors that determine the type of external resorption that develops include the type and intensity of trauma, the stage of root development, and the pulpal status (Galler et al).

### Pressure-induced resorption

Pressure both damages the cementum and provides a continuous stimulus for the resorbing cells. The most common example of this type of resorption is root resorption caused by excessive forces of orthodontic tooth movement (Fig 13-11), known as orthodontically induced inflammatory root resorption (OIIRR). Other examples are resorption caused by impacted teeth and tumors or excessive occlusal loading.

Pressure resorption has been assumed to be external, but this is not necessarily true. In orthodontics, for example, the process takes place at the apex of the tooth near the cementodentinal junction. Because the force affects the apical root, either the cementum or predentin may be damaged, so it is not unusual to see radiographic evidence of internal apical resorption in the active stage of the process.

In 1997, Bender et al termed this type of resorption *periapical replacement resorption* and offered a hypothetical explanation for it, suggesting that pulp neuropeptides may be involved in periapical replacement resorption in both vital and endodontically treated incisors (Bender et al). Their review indicated that apical resorption is significantly less frequent and less severe in endodontically treated incisors than in untreated teeth. These findings are consistent with other studies that found that endodontically treated teeth were less likely to undergo resorption during orthodontic therapy (Hamilton and Gutmann, Esteves et al).

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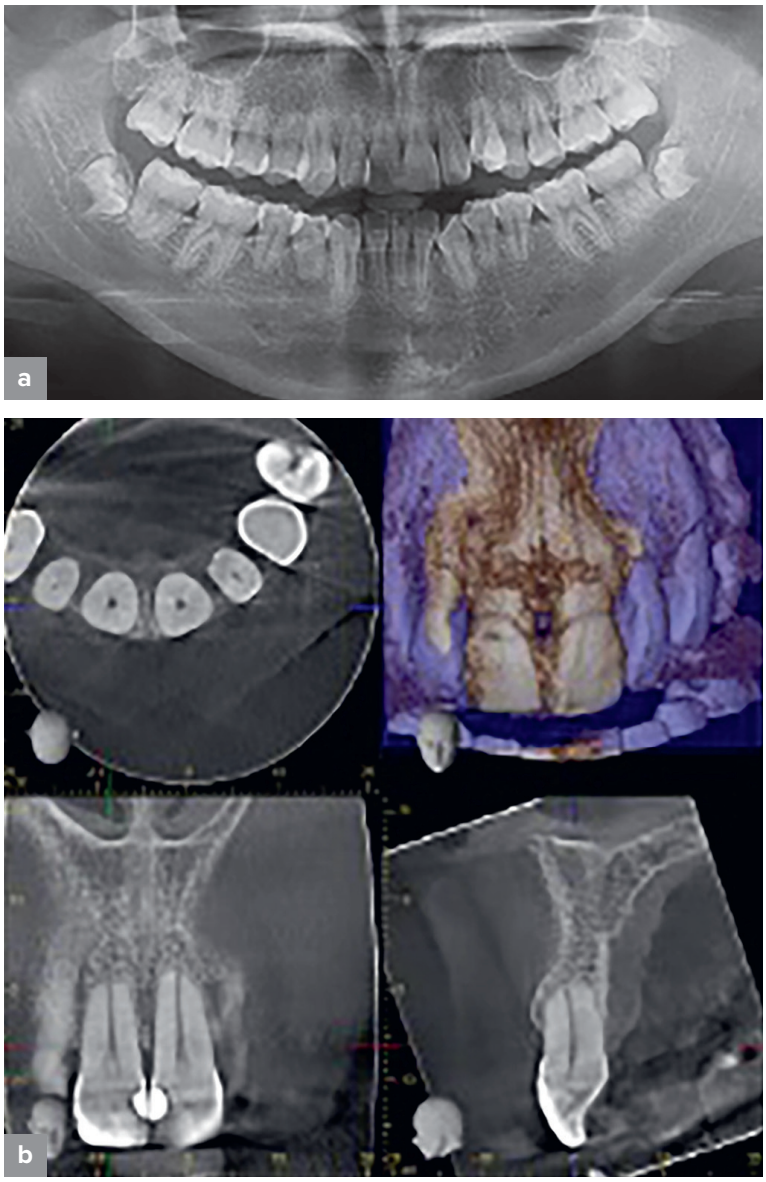


◀ **FIG 13-11** Root resorption during active orthodontic tooth movement. *(a)* The mandibular central incisors show external and internal apical resorption. (Reprinted with permission from Sameshima and Iglesias-Linares.) *(b)* Small-field CBCT of the central incisors revealing OIIRR with a compromised crown-root ratio.

OIIRR has been attributed to both treatment- and patient-related factors (Hamilton and Gutmann, Brezniak and Wasserstein, Pizzo et al, Abuabara, Lopatiene and Dumbravaite, Samandara et al). It can result in abnormal shortening of the root (see Fig 13-10). Treatment-related factors include the duration of treatment, the magnitude of force exerted, the direction of tooth movement, and the method of movement (Segal et al). Prolonged orthodontic treatment may result in an increased incidence and severity of apical root resorption (Apajalahti and Peltola). The use of intermittent forces was found to produce less OIIRR than the use of continuous forces (Ballard et al). In one study, root resorption continued for 4 weeks after the cessation of orthodontic treatment, although the use of light forces produced less root resorption and better repair than the use of heavy forces (Cheng et al, Paetyangkul et al).

The distance of movement also has an impact on OIIRR; movement over greater distances increases the probability of OIIRR (Fox). Intrusive movements and lingual root torque tend to be associated with higher levels of root resorption because they concentrate forces at the apex (Costopoulos and Nanda, Parker and Harris). Bodily tooth movement, extrusion, and lingual tipping movement, by contrast, result in a lower incidence, presumably because the stress is evenly distributed over a larger area of the root during these movements (Pizzo et al).


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In addition to factors relating to the mechanics of tooth movement, there are specific patient-related risk factors for OIIRR. Patients who have short roots prior to orthodontic treatment or who show signs of root resorption before treatment are at higher risk for OIIRR (Abuabara, Levander et al). Gender, age, systemic factors, tooth type, and morphology have been proposed as risk factors (Samandara et al, Levander et al). In addition, there is evidence for a genetic predisposition to OIIRR (Kalra et al). A link has been made between a polymorphism in the IL-1 gene (*IL1B* allele 1) and the occurrence of OIIRR. Patients who are homozygous for *IL1B* allele 1 have a 5.6-fold greater risk for OIIRR (Al-Qawasmi et al 2006). The *TNFRSF11A* gene that encodes for a TNF receptor involved in osteoclastogenesis represents another candidate gene for OIIRR (Al-Qawasmi et al 2004).

During treatment, immunoassays on the crevicular fluid of orthodontic patients have been proposed for the detection, confirmation, or cessation of OIIRR. ELISA analysis has shown higher levels of dentin sialoprotein and dentin phosphophoryn in crevicular fluid in patients with OIIRR (Mandour et al). Increased levels of these proteins appear to be a biomarker for the presence and intensity of OIIRR and may prove helpful in orthodontic clinical decision-making (Balducci et al).

The incidence of OIIRR in patients undergoing orthodontic movement is 2% to 5% (Hartsfield et al). If OIIRR is detected during active orthodontic treatment, it is recommended that treatment be halted for 2 to 3 months with passive archwires and bite. Severe OIIRR may require cessation of orthodontic treatment indefinitely (Luther et al). Other interventions including anti-inflammatory agents, lithium chloride administration, photobiomodulation with lasers, and the use of mesenchymal stem cells have also been proposed to treat OIIRR (Ueda-Ichinose et al, Ino-Kondo et al, Kirschneck et al, Gul Amuk et al, Nayyer et al).

#### Infection-induced external root resorption

Pulp space infection represents the second general type of inflammatory stimulus that can cause external root resorption. Pulp space infection can lead to external root resorption in either the apical or lateral regions of the root. The classic example of pulp space infection causing apical external root resorption is apical periodontitis with apical root resorption. The etiology for this form of apical external root resorption is infection of the root canal system. The pulp of the tooth may become necrotic for many reasons such as caries, trauma, cracks, and leaky restorations. When the pulp defenses are overwhelmed, the pulp undergoes necrosis and becomes infected, followed by diffusion of microbes and their byproducts into the surrounding periodontium. In most routine cases (nontraumatic), the root surface is protected by its intact cementum, and communication will be primarily through apical foramina or accessory canals.

Invariably, the periodontal inflammation is accompanied by resorption of the root at the cementodentinal junction. This resorption is usually not apparent radiographically but is routinely visible upon histologic evaluation. It is not obvious why the apex of the root is not as well protected from the resorbing factors produced during the inflammatory response as are the other areas of the root. A simple explanation might be that inflammation is confined to a small area at the apex of the root so that the concentration of resorbing factors is so high that the resistance of the root to resorption is overcome. Another speculation is that the cementodentinal junction at the apical foramen provides an extremely thin protective layer compared with the other areas of the root. It is also possible that the cementum and dentin could fail to coalesce in a certain percentage of cases, similar to the gap configuration of the cementoenamel junction. This allows exposure of mineralized tissue, permitting attachment of the clastic resorbing cells as previously described.

There are no clinical manifestations because apical root resorption is asymptomatic. Symptoms that might lead to its diagnosis are associated with periapical inflammation and not root resorption per se. The radiographic appearance, as mentioned earlier, is not detectable in most cases; however, when apparent, the radiolucencies are evident at the root tip and adjacent bone. Irregular shortening, stumping, or thinning of the root tip is sometimes observed (Fig 13-12a).

The histologic appearance of the soft tissue in a periapical lesion may be either granulomatous or cystic (Lalonde and Luebke) (Fig 13-12b). On the root surface, resorption of the cementum and dentin results in a scalloped appearance of the root end. Attempts at repair are often evidenced by the presence of the resorption lacunae, with resorptive and mineralization processes ongoing and adjacent to each other.

The first line of treatment for inflammatory root resorption is to prevent it. An effective way to prevent pulp space infection is to maintain the vitality of the pulp. If the pulp remains vital, the canal will be free of bacteria, and external inflammatory root resorption will not occur. Depending on the injury, overly aggressive treatment should be avoided if pulp vitality can be maintained. Certain injuries, however, have been shown to cause pulp necrosis with high certainty in mature teeth. Avulsion, intrusion, or extreme displacement of luxated mature teeth requires endodontic intervention within 2 weeks of the injury, as pulp necrosis is likely (Krastl et al 2021, Bastos et al).

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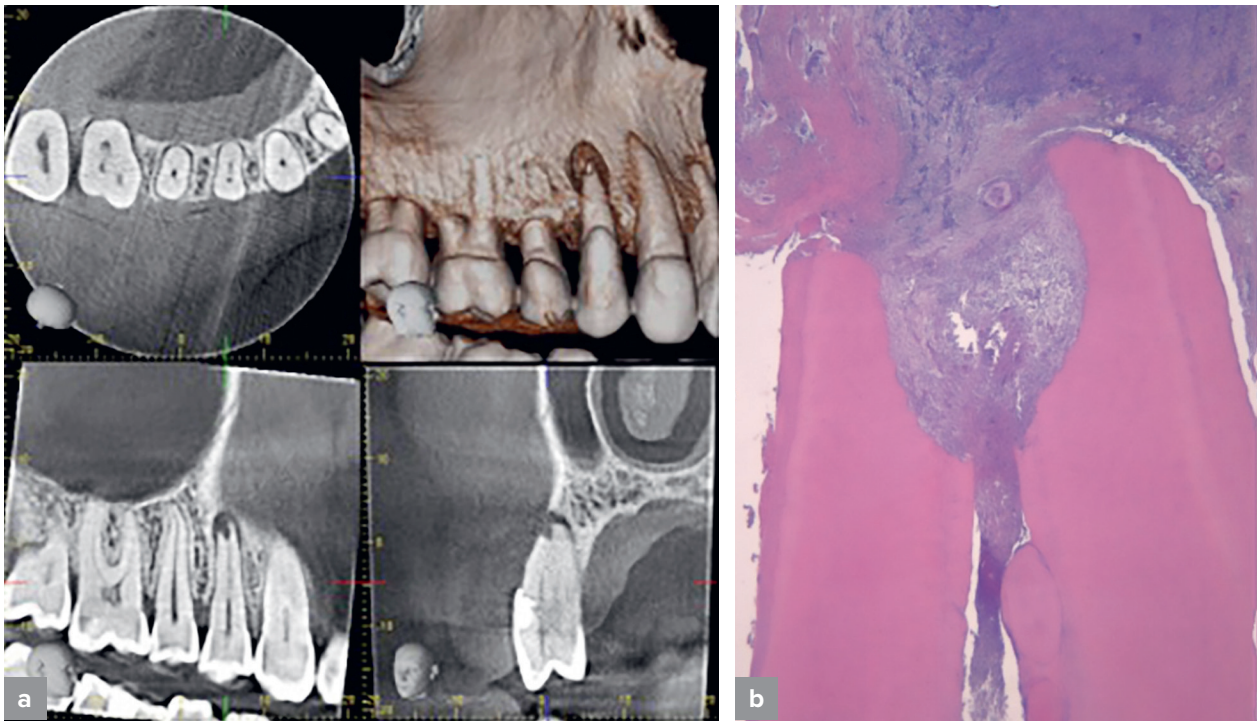


▲ **FIG 13-12** *(a and b)* Radiographic and histologic appearance, respectively, of apical resorption caused by an infected root canal. Chronic inflammation is present apically. Resorption of the external and internal aspects of the root can be seen (h&e stain; original magnification ×100). (Reprinted with permission from Ricucci et al.)

The clinician should ideally evaluate pulp space infection 7 to 10 days after an injury (Bourguignon et al, Fouad et al). Pulp sensibility test responses can often be delayed after trauma, but radiographic signs of inflammatory root resorption mandate swift endodontic intervention (Bastos et al). In cases where external root resorption with apical periodontitis is detected, treatment should be directed at removing the stimulus for the underlying inflammatory process (ie, the microbes in the root canal system) (Dalton et al, Shuping et al). If a thorough disinfection protocol is followed, a high success rate can be expected (Bystrom et al, Sjögren et al). At present, the traditional treatment protocol entails complete debridement of the root canal system with sodium hypochlorite irrigation at the first visit. Calcium hydroxide is then placed. After the intracanal medicament has been in place for at least 7 days, the root canal system is sealed. When extensive resorption is present apically, the clinician should account for canal-altering pathologic processes (apical enlargement due to resorption as well as the presence of apical periodontitis) when establishing a working length for canal debridement. Newer methods for canal disinfection such as the use of energized irrigation may offer promise in treating these cases in a single appointment, but presently outcome studies are lacking.

Attempts should be made to debride the full length of the remaining canal while an apical constriction is preserved, and minimal dentin should be removed to place the root fill. Apical closure techniques with long-term calcium hydroxide treatment or short-term barriers (eg, bioceramics and collagen plugs) may also be used, particularly if the apex is large or open, to ensure a better prognosis for nonsurgical endodontic therapy (Kerekes et al, Simon et al). If nonsurgical therapy has been unsuccessful in arresting the resorption, apical surgery should be considered.

Radiographically, inflammatory root resorption is observed as progressive radiolucent areas of the root and adjacent bone (Fig 13-13a). This contrasts with ERR, where the tooth profile is irregular but there are no associated areas of low density indicative of bone loss (Fig 13-13b).

Following severe injuries where vitality has been lost, it is possible under some circumstances to promote revascularization of the pulp. Such revascularization is possible in young teeth with incompletely formed apices if the teeth are replaced in their original position within 60


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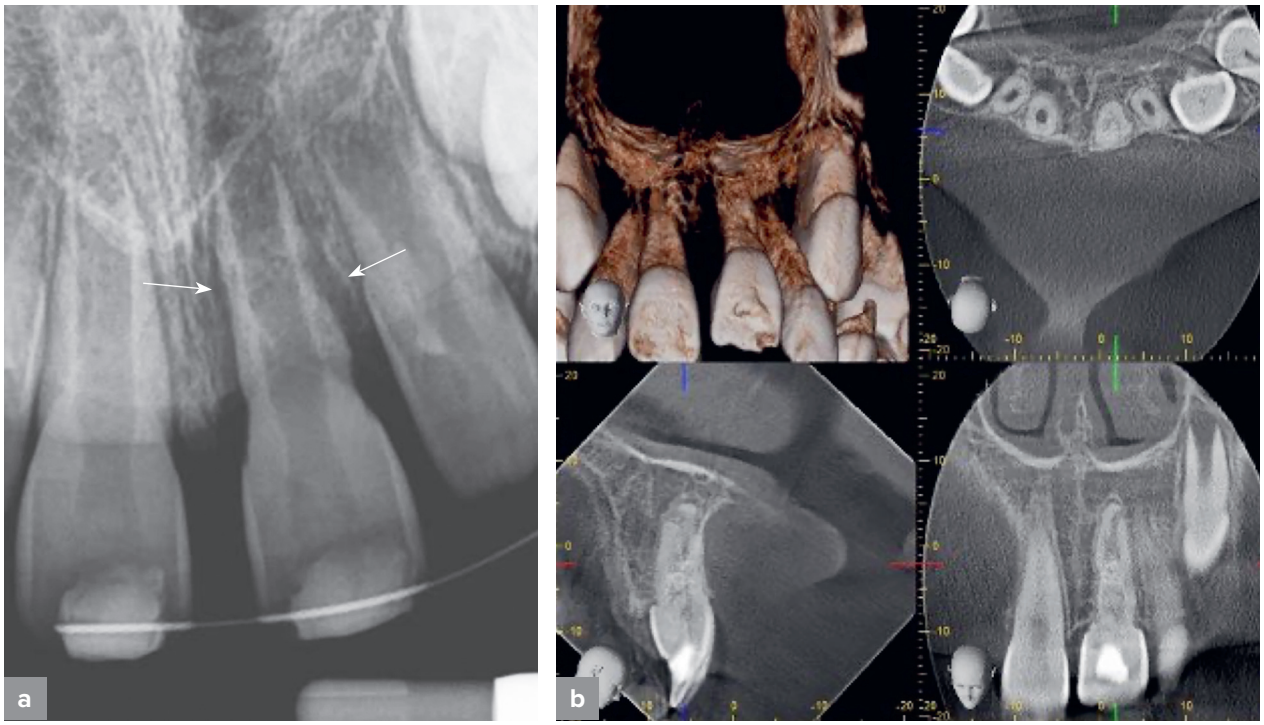


▲ **FIG 13-13** *(a)* Radiographic appearance of external inflammatory resorption caused by pulpal infection. Note the radiolucencies on the root surface and adjacent bone *(arrows). (b)* The results of revascularization of the same tooth. Root formation continued, but replacement resorption ensued, necessitating decoronation of the tooth in this young patient.

minutes of the injury (Cvek et al). If the tooth has been avulsed, soaking it in doxycycline for 5 minutes before reimplantation has been shown to double the revascularization rate. However, even under the best conditions, revascularization will occur only about 50% of the time, which poses a diagnostic challenge. If the pulp revascularizes, external root resorption will not occur, and the root will continue to develop and strengthen. However, if the pulp becomes necrotic and infected, the subsequent external inflammatory root resorption that develops could result in the loss of the tooth in a very short time. Frequent monitoring of the case as per recommended guidelines for a specific injury can minimize the risk of extensive progression of inflammatory root resorption (Krastl et al 2021).

Regenerative techniques have been used in necrotic traumatized teeth to effect revitalization. Studies have reported a high rate of success with immature traumatized teeth. One prospective cohort study reported a 60% success rate for pulp revitalization in immature traumatized teeth. Failures were largely due to a failure to obtain a satisfactory blood clot and persistent infection (Wikström et al). Damage to the cementum, the apical papilla, and HERS are factors that the authors speculate are theoretical reasons for a lower success rate for regeneration when compared to immature necrotic teeth without trauma. Regeneration techniques for mature teeth have also been reported and offer promise for the treatment of traumatized teeth with closed apices (Youssef et al, Arslan et al, Glynis et al).

At present, the diagnostic tools available cannot detect pulp vitality until approximately 6 months following successful revascularization. Waiting for a response to pulp sensibility tests to occur is therefore unrealistic, and healing should be monitored radiographically to ensure that resorption or apical periodontitis is not present and that there is continued root development. Laser Doppler flowmetry has been shown to be an excellent diagnostic tool for the detection of revascularization in immature teeth. This tool appears to accurately detect the presence of vital tissue in the pulp space by 4 weeks after the traumatic techniques (Strobl et al, Karayilmaz and Kirzioglu). Other techniques shown to improve accuracy of vital pulp detection include ultrasound Doppler flowmetry and pulse oximetry (Lima et al, Ahn et al). The clinical availability of these tools, however, is lacking.

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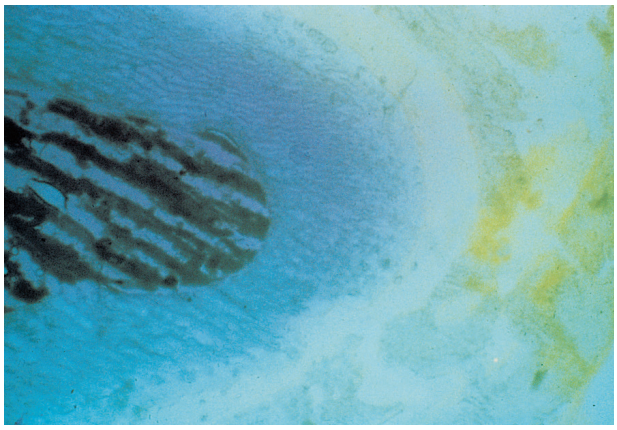
From a microbial point of view, timely treatment of necrotic but not yet infected teeth may be considered equivalent to the treatment of teeth with vital pulps. As previously mentioned, mature permanent teeth that have sustained avulsion or intrusive luxation are likely to undergo ischemic pulp necrosis, so the prevailing recommendation is immediate endodontic intervention (Krastl et al 2021). Because infection in this time frame is unlikely, endodontic treatment may be completed in one visit. Patient management issues and injuries to other structures may make complete treatment difficult so soon after a serious traumatic injury; therefore, it is beneficial to start the endodontic treatment with chemomechanical preparation, after which an intracanal dressing of calcium hydroxide is placed (Trope et al). The practitioner can then obturate the canal when periodontal healing of the injury is complete, approximately 1 month after the instrumentation visit. There appears to be no need for long-term calcium hydroxide treatment in cases where endodontic treatment is started within 10 days of the injury. However, calcium hydroxide can be applied in a compliant patient for up to 6 months to ensure periodontal health prior to root canal filling with gutta-percha.

When root canal treatment is initiated later than 10 days after trauma or when active external inflammatory resorption is observed, the preferred antibacterial protocol consists of chemomechanical preparation followed by long-term dressing with densely packed calcium hydroxide as previously described. While other medicaments have been used, calcium hydroxide creates an alkaline pH in the surrounding dentinal tubules (Fig 13-14), kills bacteria, and neutralizes endotoxin, a potent inflammatory stimulator (Farhad and Mohammadi). Unlike antibiotics or steroids, calcium hydroxide maintains its therapeutic action for an extended period of time due to its low solubility, making it more suitable for cases that require monitoring longer than a few days.



▲ **FIG 13-14** Cross section of a root filled with calcium hydroxide. The pH indicator shows that the canal and surrounding dentin are basic, while the surrounding tissue is slightly acidic.

## Invasive cervical resorption

Unlike external inflammatory root resorption, where the stimulus for destruction of the root and bone is due to inflammation or infection of the pulp, invasive cervical resorption (ICR) can—and usually does—initiate in a tooth with a healthy pulp. Pulpal involvement is only seen in the late stages of the process where the pulp canal is breached by the resorptive process. ICR originates on the external surface of the tooth immediately below the epithelial attachment. It characteristically penetrates the cementum and spreads vertically and circumferentially around the pulp chamber/canal (see Fig 13-3). The anatomical connotation of "cervical" root resorption has led to confusion and misdiagnosis of this condition, inspiring attempts to rename this type of external resorption (Heithersay 1994, Kandalgaonkar et al, Frank and Bakland, Patel et al 2010). This is due to the observation that the periodontal attachment of teeth is not always at the cervical margin; the same process can occur more apically on the root surface where there has been gingival recession. Because of this, *subepithelial inflammatory root resorption* has been proposed as a more accurate name for this process. For the purposes of this discussion, however, the term ICR will be used.

The pathogenesis of ICR is not fully understood (Heithersay 1994, Patel et al 2009b). However, because its histologic appearance and progressive nature are identical to other forms of progressive inflammatory root resorption, it seems logical that the pathogenesis would be the same (ie, an unprotected or altered root surface that attracts resorbing cells and an inflammatory response maintained by infection or trauma). Other theories propose that the process could be a type of benign proliferative fibrovascular or fibro-osseous disorder in which bacteria are only involved secondarily. ICR lesions have been examined using CBCT, nanofocus computed tomography, SEM, and hard tissue histology (Mavridou et al 2017, Vaz de Souza et al, Patel et al 2009a). The results of these studies have demonstrated the staging of the ICR process (Fig 13-15). The first stage is characterized by penetration of the resorption below the epithelial attachment. The second stage is characterized by the 3D spread of the resorption around the pulp space. The third stage represents a healing phase where osseous-like tissue is observed to proliferate into the resorptive cavity. This pattern is affected by the pulpal status of the tooth. In teeth with vital pulps, the pericanal resorption resistant sheet, which contains predentin, surrounds and protects the pulp and stops the invasion of the resorptive tissue.



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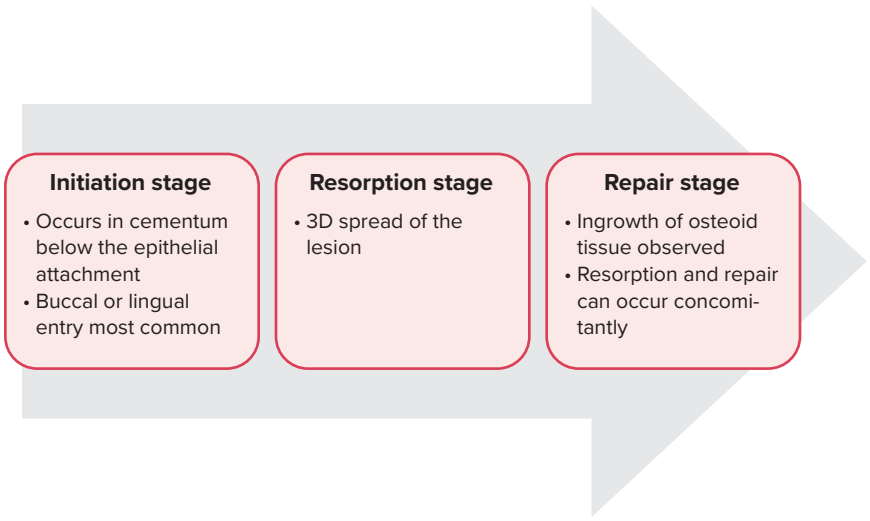

Initiation stage
• Occurs in cementum below the epithelial attachment
• Buccal or lingual entry most common
Resorption stage
• 3D spread of the lesion
Repair stage
• Ingrowth of osteoid tissue observed
• Resorption and repair can occur concomitantly

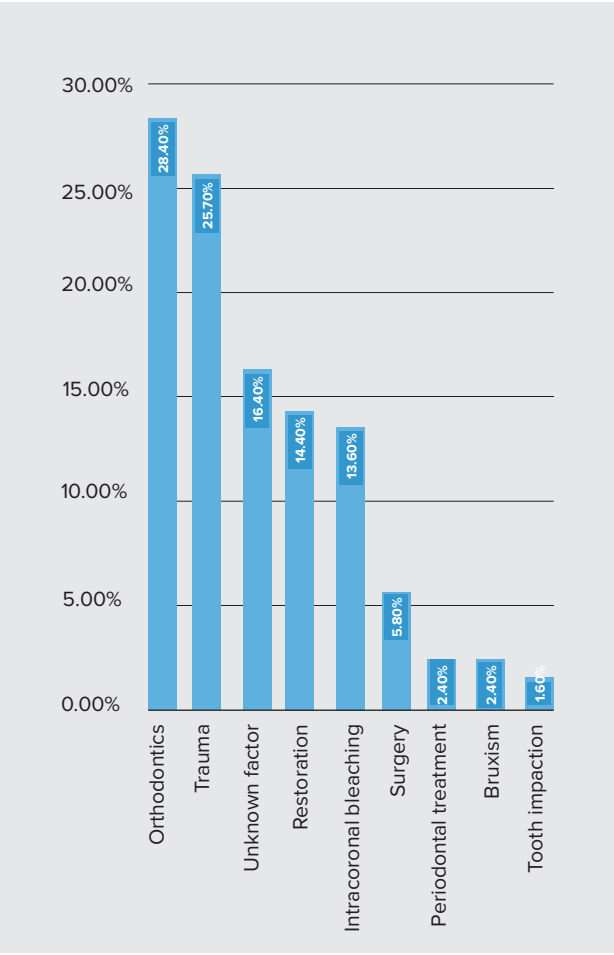
◀ **FIG 13-15** Proposed stages of ICR as observed with imaging and histology. (Data from Mavridou et al 2016.)

However, teeth that have undergone root canal therapy lack this protective sheet, and it has been observed that there is less repair and that the resorption is more aggressive in these teeth when compared to their vital counterparts (Mavridou et al 2017).

Two types of healing have been described. One shows an ingrowth of healthy bone into the resorptive defect and the other shows resumption of a healthy PDL and cementum formation. Both reparative and resorptive processes can progress concomitantly, and ultimately the dominance of one process over the other determines the fate of the tooth (Mavridou et al 2017). At this time, there are no clinical predictors for healing of ICR, thus confounding the decision to treat or to monitor.

### Etiology

Although an exact etiology has not been identified, several predisposing factors have been associated with ICR (Fig 13-16). These include trauma (both acute and chronic), orthodontics, intracoronal bleaching, dentoalveolar surgery, parafunctional habits, medications, diabetes, restorations, and periodontal disease or treatment. Most of these associated factors have the potential to damage cementum and create a hypoxic microenvironment, which induces inflammatory mediators—factors that are known to stimulate osteoclastogenesis and provide a mineralized substrate for binding (Irinakis et al 2020). The dental pulp does not appear to play a direct role and is usually normal in these cases unless exposure results from the resorptive process. Because the source of stimulation is not the




▲ **FIG 13-16** Predisposing factors for ICR. (Data from Chen et al 2021.)

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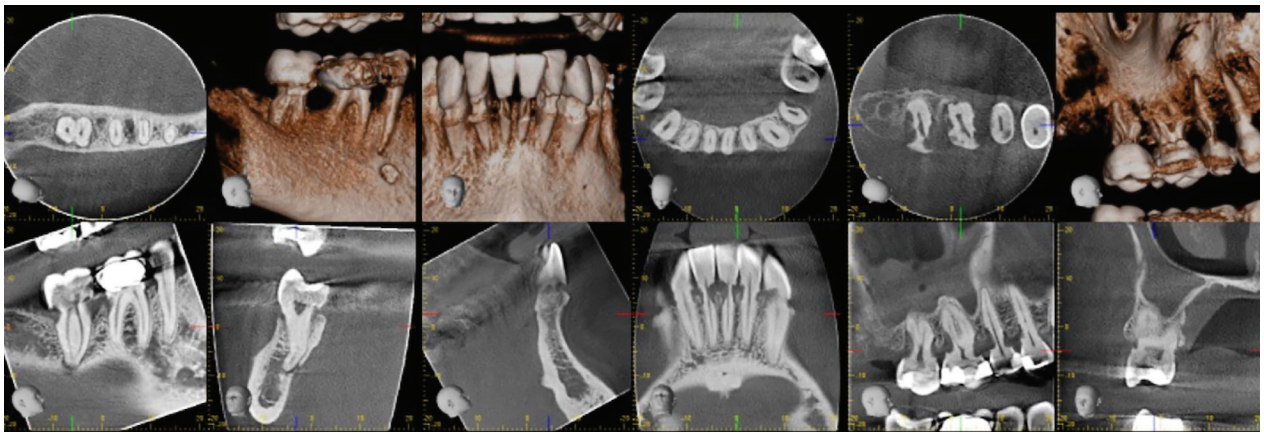

▲ **FIG 13-17** Radiographs showing multiple resorptive lesions that arose after cessation of denosumab treatment for osteoporosis.

◀ **FIG 13-18** Granulation tissue from the coronal spread of ICR results in the undermining of enamel and the appearance of a pink spot.

pulp, it has been postulated that bacteria or viruses in the sulcus of the tooth stimulate and/or sustain an inflammatory response in the periodontium at the attachment level of the root (von Arx et al, Patel et al 2016b, Kumar et al). Recent studies have provided evidence that there is a genetic component to the susceptibility to ICR (Neely et al). Whole exome sequencing of a family with multiple members who developed ICR revealed a mutation in the interferon regulatory factor 8 gene *(IRF8)*. This gene is known to facilitate osteoclastogenesis. Mice lacking *IRF8* expression show increased osteoclast activity in the alveolar bone surrounding their molars (Thumbigere-Math et al). In the majority of cases, it has been shown that more than one associated factor is present in patients with ICR, confirming that it is likely multifactorial (Mavridou et al 2017).

A particularly concerning association with ICR is the use of denosumab, a medication prescribed for osteoporosis and some other forms of osteolytic disease (Alyahya and Myers). Denosumab is a human monoclonal antibody that neutralizes RANKL and inhibits osteoclastogenesis. It is an effective anti-osteoporosis medication. In a case-control study, it was determined that denosumab was significantly associated with the occurrence of ICR. Numerous case reports have been presented where patients who have discontinued denosumab therapy have a rebound effect that results in rapid bone loss and multiple ICR lesions (Mikuskova et al, Beaumont et al, Alyahya and Myers, Deeb et al). This rebound effect regarding bone density is so profound in osteoporosis patients that a drug holiday is not an option with this drug (McDonough et al, Herath et al). Dental patients should be counseled about the use of this medication and the possibility of tooth loss once the medication is discontinued (Fig 13-17).

In terms of tooth type involved in ICR, maxillary anterior teeth are the most commonly affected, followed by maxillary premolars. Maxillary teeth in general appear to be more commonly involved when compared to mandibular teeth (76.19% versus 23.81%) (Jeng et al).

ICR is asymptomatic and is usually detected only through routine radiographs. Occasionally, symptoms of pulpitis will develop if the resorption is extensive. Clinically, it can be detected in some cases by a subgingival cavitation that is hard when probed with an explorer and may bleed easily due to the highly vascular nature of the resorptive tissue. When the resorption is long-standing, granulation tissue may undermine the enamel of the crown of the tooth, resulting in a pinkish appearance. This "pink spot" has traditionally been used to describe the pathognomonic clinical picture of internal root resorption, leading to the misdiagnosis and treatment of many cases of ICR as internal root resorption (Fig 13-18).


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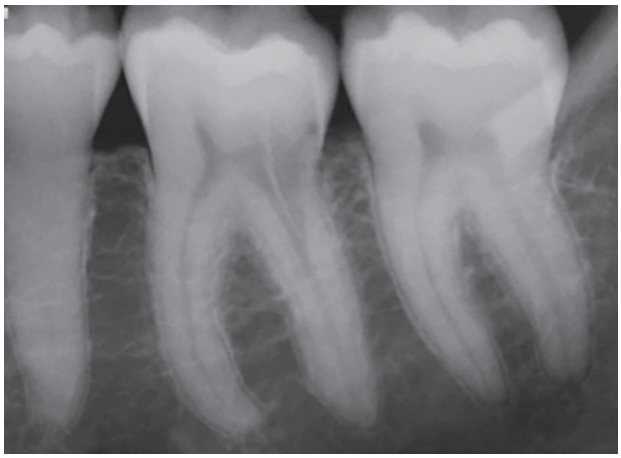

▲ **FIG 13-19** A periapical radiograph shows ICR on the distal of the mandibular left first molar. Note the mottled appearance and the canal outline that remains present.

▲ **FIG 13-20** Comparison between conventional 2D radiography and CBCT. *(a)* The periapical radiograph suggests a resorptive lesion on the palatal root. *(b)* CBCT imaging of the same tooth, with slices through the palatal root, shows an extensive resorptive lesion that has undermined and destroyed a large amount of tooth structure.

### Radiographic appearance

The 2D radiographic appearance of ICR can be quite variable. If the resorptive process occurs mesially or distally on the root surface, small radiolucent openings into the root are common. The radiolucency expands coronally and apically in the dentin and reaches, but usually does not perforate, the root canal. If the resorptive process is buccal or palatolingual, the radiographic picture is dependent on the extent to which the resorptive process has spread in the dentin. It can be seen as a radiolucency at the attachment level or as mottled or mixed radiolucencies if it has spread coronally or apically to any considerable degree. Because the pulp is not involved, the canal outline can usually be distinguished through the resorptive defect (Fig 13-19). CBCT technology has been utilized to allow more accurate assessment of the location and extent of ICR (Patel et al 2009a, Cohenca et al 2007, Bhuva et al). It has been shown to have higher sensitivity, specificity, and accuracy than conventional 2D radiography in the assessment of ICR lesions (Vaz de Souza et al, Goodell et al) (Fig 13-20). Studies have shown that consistency in classification and treatment planning are improved with CBCT when compared to 2D imaging (Matny et al). Both the European Society of Endodontology and the American Association of Endodontists (AAE) and American Academy of Oral and Maxillofacial Radiology (AAOMR) joint position statements identify CBCT as the imaging modality of choice for the evaluation and treatment of resorptive lesions, including ICR (Patel et al 2019, AAE/AAOMR).

Historically, a classification scheme based on 2D radiographs was proposed for the evaluation of and treatment planning for ICR (Heithersay 1994, Heithersay 1999) (Fig 13-21 and Table 13-2). It was based on the extent of invasion and has been correlated with outcomes in one clinical study (Heithersay et al). Because it is based on 2D radiographs, a limitation of this classification scheme is that it only accurately evaluates the interproximal spread of the resorption but cannot accurately assess the proximity to the pulp or the circumferential spread (Patel et al 2018b). Attempts have been made to correlate the Heithersay system with CBCT data (Matny et al). More recent studies have used CBCT to evaluate and classify

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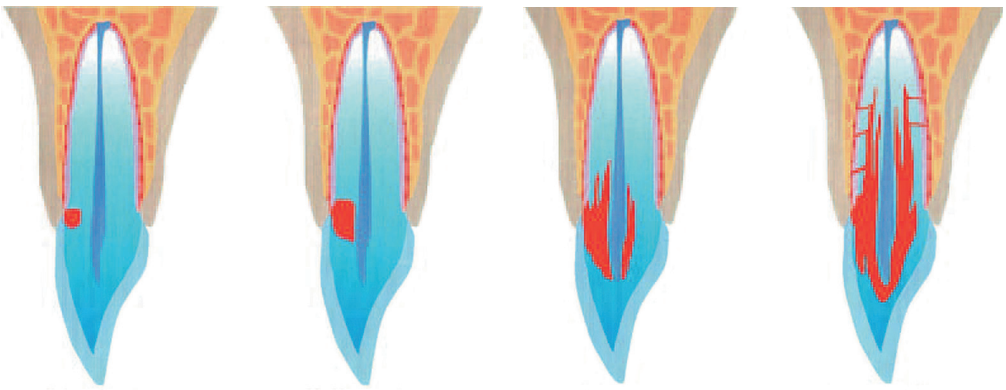


◀ **FIG 13-21** Heithersay's clinical classification of ICR based on 2D radiography. (Reprinted with permission from Heithersay 1999.)

**TABLE 13-2** Heithersay's classification of ICR lesions based on 2D imaging

| Class | Description |
|---|---|
| 1 | Small invasive lesion near the epithelial attachment of the tooth and shallow penetration into dentin |
| 2 | Well-defined lesion that has penetrated just below the epithelial attachment into the coronal dentin but exhibits no extension into radicular dentin |
| 3 | Deeper invasion of the lesion into the coronal third of the root |
| 4 | Large, invasive lesion that extends beyond the coronal third of the root |

Data from Heithersay 1999.

**TABLE 13-3** Patel's clinical classification of ICR lesions based on CBCT

| Class | Height | Circumferential spread | Proximity to root canal |
|---|---|---|---|
| 1 | At CEJ or coronal to crestal bone | A: ≤ 90 degrees | d: Lesion confined to dentin |
| 2 | Extends into coronal third of root and apically to crestal bone | B: > 90 to ≤ 180 degrees | p: Probable pulpal involvement |
| 3 | Extends into middle third of root | C: > 180 to ≤ 270 degrees | |
| 4 | Extends into apical third of root | D: > 270 degrees | |

Data from Patel et al 2018b.

cervical resorptive lesions based on small-field, high-resolution scans. One study utilized lesion height as well as circumferential spread to propose an updated classification scheme to guide the assessment and treatment planning for these lesions (Patel et al 2018b) (Table 13-3).

### Treatment

Treatment for ICR relies on the removal of the resorptive tissue and restoration of the defect with a suitable material. The outcome of treatment appears to be directly related to the extent of the resorptive process at the time of intervention and the tooth location (Irinakis et al 2022). Therefore, early intervention improves the prognosis. A variety of treatment approaches have been suggested for treating ICR lesions. These include external repair of the resorptive defect with or without endodontic intervention (depending on the proximity of the lesion to the pulp), endodontic treatment and internal repair of the resorptive defect, intentional replantation, periodic monitoring for teeth that are not amenable to treatment, and extraction (Patel et al 2018a, Patel et al 2016a, Baranwal, Tsaousoglou et al). A correlation between the classification and the treatment outcome using either the Heithersay classification system or more recent systems based on 3D image assessment shows an inversely proportional success rate compared to lesion size (ie, the smaller the lesion, the greater the success rate) (Patel et al 2018a, Schwartz et al, Mavridou et al 2022, Patel et al 2018b, Heithersay 1999). Hence, early detection is critical to successful treatment outcomes in ICR lesions.


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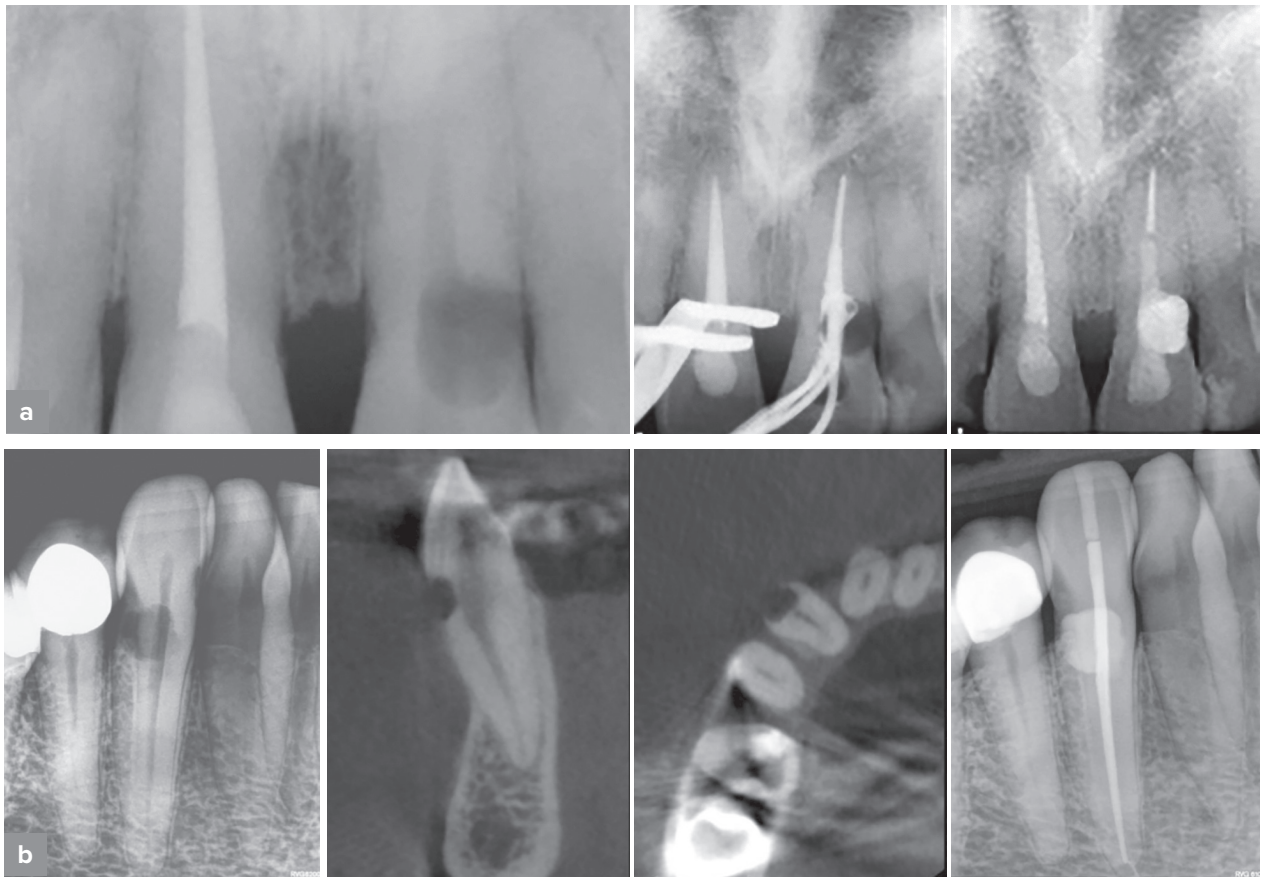
External treatment of the ICR lesion entails removal of the granulomatous tissue until no bleeding points can be detected. This can be done with a bur or excavator. It has been shown that failure to remove all of the resorbing tissue lowers the prognosis for treatment (Patel et al 2009b). Additional treatment with 90% trichloroacetic acid (TCA) or 3% to 5% sodium hypochlorite has been shown to aid in the removal of tissue that may be difficult to see or access (Heithersay 1994, Patel et al 2018a). Case reports have maintained that Er:YAG lasers can also be utilized for tissue removal (Kirmizi et al). Once the tissue is removed, the defect should be restored. The material chosen for the restoration is dependent on the location of the defect. If the defect communicates with the oral cavity, a composite resin or glass ionomer is recommended (Estevez et al) (see Fig 13-21). Subgingival repairs or repairs in close proximity to the pulp may be restored with Biodentine (Septodont) or bioceramics (Rajasekharan et al). If the dentin surface has been treated with TCA, the surface dentin is severely demineralized and must be removed with a bur before bonding (Schwartz et al). If the preoperative CBCT shows that the resorptive lesion approximates or has invaded the pulp, root canal therapy should be completed before the external repair to avoid inadvertent extrusion of restorative material into the pulp space. Alternatively, vital pulp therapy can be considered depending on the case (Asgary et al).

Internal repair of a resorptive defect may be elected if the lesion is extensive and external access is not possible. If there are symptoms of irreversible pulpitis or apical periodontitis, or if the preoperative scan indicates pulpal involvement, root canal therapy should be completed first. Subsequently, the ICR lesions should be accessed from within the root canal system with a bur and the granulomatous tissue removed through a combination of mechanical and chemical means. A recommended protocol entails removal of the granulomatous tissue with a carbide bur followed by treatment with 90% TCA. Alternatively, 5% sodium hypochlorite can be used. This sequence can be repeated until all bleeding has ceased and all tissue is removed. At that point, the resorptive defect can be restored with a resin composite, glass ionomer, or bioceramic material if the lesion is subgingival. Long-term calcium hydroxide has proven to be effective in chemically cauterizing resorptive tissue and may be used in internal repairs (Mavridou et al 2022). Figure 13-22 illustrates two cases of ICR treatment.




▲ **FIG 13-22** *(a and b)* Two cases that illustrate the treatment sequence for an ICR defect that approximates the pulp space. (Part *b* courtesy of Dr Richard Schwartz.)

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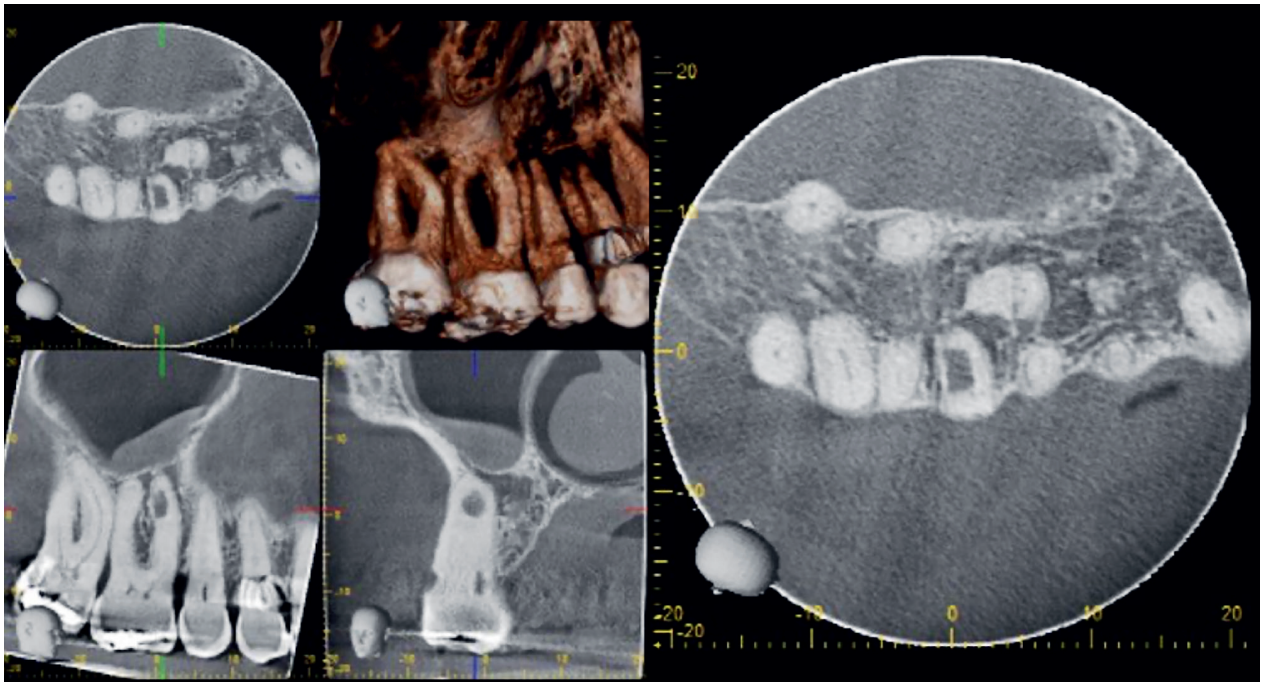

▲ **FIG 13-23** Intraradicular internal resorption seen on the apical aspect of the mesial root of a maxillary molar. Note that the axial slice through the resorptive lesion does not show a portal of entry as you would see with ICR. ICR is also evident at the cervical aspect of the mesiobuccal root.

## Internal Inflammatory Root Resorption

Internal inflammatory root resorption is a rare phenomenon that occurs in response to infection and responds favorably to conventional treatment. There are two recognized forms of internal root resorption: internal inflammatory resorption and internal replacement resorption. The former is characterized by resorption of dentin and replacement with granulation tissue, and the latter is characterized by resorption of dentin and replacement with tissue that resembles bone. The prevalence has been estimated to be between 0.01% and 55% depending on the method used for detection (radiographic versus histology or SEM) (Gabor et al). Internal inflammatory resorption is further classified according to location as apical or intraradicular (see Table 13-3). Apical internal inflammatory root resorption has been shown to be a common occurrence in teeth with periapical pathosis (Vier and Figueiredo). The majority of teeth with periapical pathosis demonstrate some degree of apical internal resorption when analyzed with SEM (Gabor et al). These lesions may not be apparent clinically; nevertheless, the clinician should presuppose their presence. Apical internal inflammatory resorptive lesions can contribute to the observed inaccuracies of electronic length determination in teeth with large apical lesions. Intraradicular internal resorption is identified as a round or oval lesion contained within the root canal (Fig 13-23).

Conventional endodontic therapy will stop progression of the lesion. Early intervention can prevent perforation of the periodontium, which can complicate clinical management.

### Etiology

Internal root resorption is marked by resorption of the internal aspect of the root via multinucleated giant cells adjacent to granulation tissue in the pulp (Lyroudia et al) (Fig 13-24). Chronic inflammatory tissue is common in the pulp, but only rarely does it result in the conditions necessary for recruitment and activation of the clastic cells that mediate resorption. The exception to this is vital pulp therapy in young carious teeth, where 88.2% of failures were attributed to internal resorption (Sonmez and Duruturk).

There are different hypotheses regarding the origin of the pulpal granulation tissue involved in internal resorption. The first and most widely accepted hypothesis is that infected coronal pulp tissue leads to the formation of adjacent apical granulation pulp tissue. A second hypothesis proposes that the granulation tissue is of nonpulpal origin, possibly originating from cells circulating in the vascular compartment or from cells originating in


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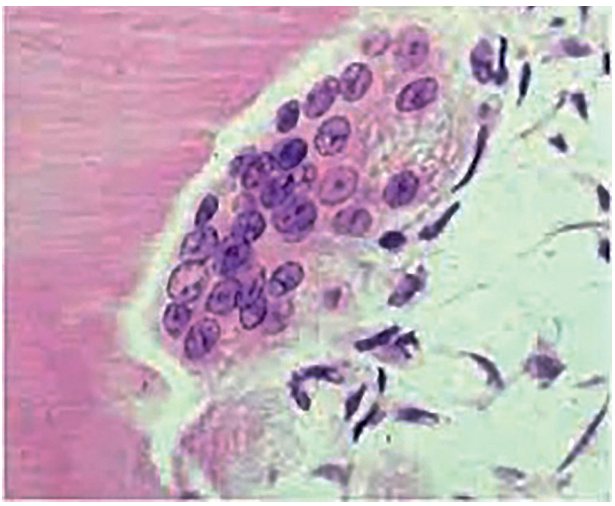

▶ **FIG 13-24** Histologic section of internal root resorption. Odontoclasts can be seen adjacent to resorbed dentin. (Reprinted with permission from Haapasalo.)

the periodontium. It has been shown that communication between the coronal necrotic tissue and the vital pulp is through appropriately oriented dentinal tubules (Wedenberg and Zetterqvist). One investigation reported that resorption of the dentin is frequently associated with deposition of hard tissue resembling bone or cementum, not dentin (Cvek et al, Allen and Gutmann). The investigators postulated that the resorbing tissue is not of pulpal origin but is actually "metaplastic" tissue derived from pulpal invasion by macrophage-like cells circulating in the vascular compartment. Still others have proposed that the pulp tissue is replaced by periodontium-like connective tissue when internal resorption is present (Stanley). These hypotheses are not mutually exclusive, and it is possible that all contribute to the formation of granulation tissue in pulp in various clinical cases.

Even though there may be granulation tissue in pulp, internal root resorption takes place only when the odontoblastic layer and predentin are lost or altered (Trope). Causes of predentin loss adjacent to granulation tissue are not obvious, but trauma has been frequently suggested as an initiating factor in internal resorption (Patel et al 2010, Wedenberg and Zetterqvist). The dynamics of internal resorption are divided into two types: transient and progressive, the latter of which requires continuous stimulation by infection.

Another possible cause of predentin loss may be extreme heat produced by cutting of dentin without adequate water spray. The heat presumably destroys the predentin layer, predisposing the tooth to internal resorption if there is continued inflammatory stimulation of the coronal pulp. Under these conditions, bacteria and their byproducts initiate inflammation, thereby stimulating resorbing giant cells in the vital pulp adjacent to the denuded internal root surface. In support of this possible scenario, internal root resorption has been produced experimentally by the application of diathermy (Gottlieb and Orban).

## Clinical manifestations

Internal root resorption is usually asymptomatic and is first recognized clinically through routine radiographs. Pain may be a presenting symptom if perforation of the coronal aspect of the tooth occurs and the granulation tissue is exposed to oral fluids. For internal resorption to be active, at least part of the pulp must be vital so that a positive response to pulpal sensitivity testing is possible. The coronal portion of the pulp is often necrotic, whereas the apical pulp, which includes the internal resorptive defect, may remain vital. Therefore, a negative sensitivity test result does not rule out active internal resorption. It is also possible that the pulp will become nonvital after a period of active resorption, resulting in a negative sensitivity test as well as radiographic signs of internal resorption and/or apical inflammation.

Traditionally, the pink tooth, resulting from the granulation tissue in the coronal dentin that undermines the coronal enamel, has been thought to be pathognomonic of internal root resorption. However, as already discussed, a pink tooth can also be a feature of ICR, which must be ruled out before a diagnosis of internal root resorption is made (see Fig 13-18). CBCT has been shown to be valuable in making this determination (Patel et al 2009a) (see Fig 13-20).

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## Radiographic appearance

The usual radiographic presentation of internal root resorption is a fairly uniform radiolucent enlargement of the pulp canal. Because the resorption is initiated in the root canal, the resorptive defect includes some part of the root canal space. Therefore, the original outline of the root canal is distorted in 2D radiography as well as 3D imaging. Only on rare occasions when the internal resorptive defect penetrates the root and impacts the PDL does the adjacent bone show radiographic changes. While angled conventional radiographs can help to discriminate between internal and external resorptive processes, CBCT has been shown to be superior to conventional radiography in the detection of resorptive lesions and is the modality of choice for the detection and assessment of internal root resorptive lesions (Patel et al 2009a, AAE/AAOMR).

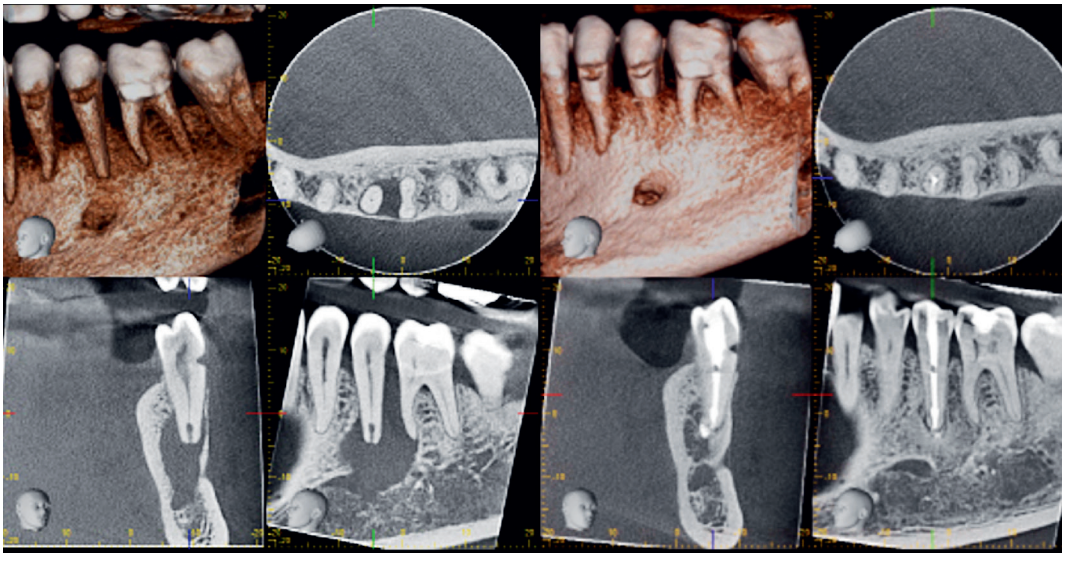
## Histologic appearance

Like that of other inflammatory resorptive defects, the histologic presentation of internal resorption is one of granulation tissue with multinucleated giant cells. An area of necrotic pulp is present coronal to the granulation tissue. Dentinal tubules that contain microorganisms and communicate between the necrotic zone and the granulation tissue are sometimes visible. Unlike the bone in external root resorption, the adjacent bone is not affected by internal root resorption. Internal replacement resorptive lesions can show tissue resembling periodontal connective tissue with osteogenic potential that gives rise to hard tissue that resembles cementum or bone (Allen and Gutmann). It has been theorized that this type of internal resorption is reflective of a response to low-grade, chronic irritation (Wedenberg and Zetterqvist).

## Treatment

Treatment of internal root resorption is straightforward and predictable. Because the resorptive defect is the result of the inflamed pulp and the blood supply to the tissue is through the apical foramina, endodontic treatment that effectively removes the blood supply to the resorbing cells is the treatment approach. Thorough debridement of the defect can pose challenges, particularly if the lesion has perforated. After adequate anesthesia is obtained, the canal apical to the internal defect is explored and a working length determined. The apical canal should be thoroughly instrumented to ensure that the blood supply to the tissue resorbing the root is eliminated. This is accomplished with a combination of instrumentation and active/energized irrigation with sodium hypochlorite (Nasab Mobarakeh et al, Yargici and Kaptan, Nilsson et al).

When root canal instrumentation is completed, paper points should be able to maintain a blood-free, dry canal unless the resorptive defect has perforated the canal wall. Calcium hydroxide can be placed in the canal to facilitate complete removal of the tissue in the irregular defect at a subsequent visit when the tooth and defect are sealed. In the instance of a resorptive perforation, the defect can be sealed with a bioceramic putty or Biodentine (Pereira da Costa et al). The use of a barrier technique may be necessary in large apical resorptive defects (Khetarpal et al, Pereira da Costa et al, Juez et al). Likewise, thermoplasticized filling techniques work best to fill the irregular canal morphology created by the resorption (Fig 13-25).



◀ **FIG 13-25** Apical internal root resorption with associated apical periodontitis. Thorough cleaning of the root canal and obturation with a thermoplasticized filling technique has stopped the resorption and resulted in osseous healing.



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## Diagnostic Features of External and Internal Root Resorption

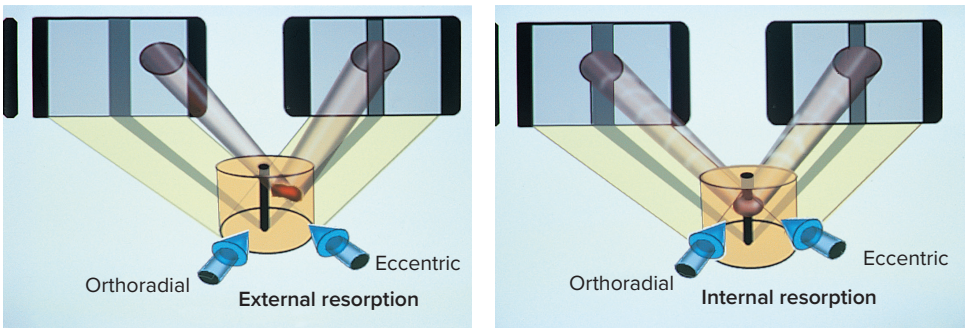
It is often very difficult to distinguish external from internal root resorption, so misdiagnosis and incorrect treatment can result. This section discusses typical diagnostic features of each type of resorption. Table 13-4 provides an overview of the key elements of these diagnostic features and may serve as a quick reference for clinical practice.

### Radiographic features

When confined to 2D radiography, a change of x-ray angle can give an indication of whether a resorptive defect is internal or external. A lesion of internal origin appears close to the canal whatever the angle of the x-ray. On the other hand, a defect on the external aspect of the root seems to move away from the canal as the x-ray angle changes (Fig 13-26). In addition, it is usually possible to

**TABLE 13-4** Diagnostic features of root resorption

| Type of resorption | Sensibility testing | History of trauma | Radiographic presentation | Bone loss | Anatomical location | Clinical presentation |
|---|---|---|---|---|---|---|
| Apical external inflammatory root resorption due to infection | Negative | Not necessary | Likely on CBCT | Yes | Apical or adjacent to lateral/furcal canals | May be discolored, with or without pain due to infection |
| Lateral external inflammatory root resorption | Negative | Yes | Bone loss and tooth structure loss | Yes | Lateral root | May be asymptomatic or have signs of symptomatic apical periodontitis |
| Invasive cervical resorption | Positive until pulp exposure and necrosis | Yes, but may be forgotten or trauma is chronic (bruxism) | Dentin resorbed with or without bone loss (dependent on pulp status) | Crestal bone loss at portal | Defect enters at crestal bone and spreads three dimensionally | Usually asymptomatic, but patient may notice gingival soreness; pink spot possible |
| Internal root resorption | Variable | History of trauma or extensive restorative procedures | Lesion at any location along root; axial slices of CBCT show no communication with PDL (unless perforated) | No, unless necrosis and infection have occurred | Occurs at any point along the canal | May be asymptomatic or have symptoms of apical periodontitis; pink spot possible |




▶ **FIG 13-26** In 2D radiography, a change in angulation can indicate whether the resorptive defect is internal or external.

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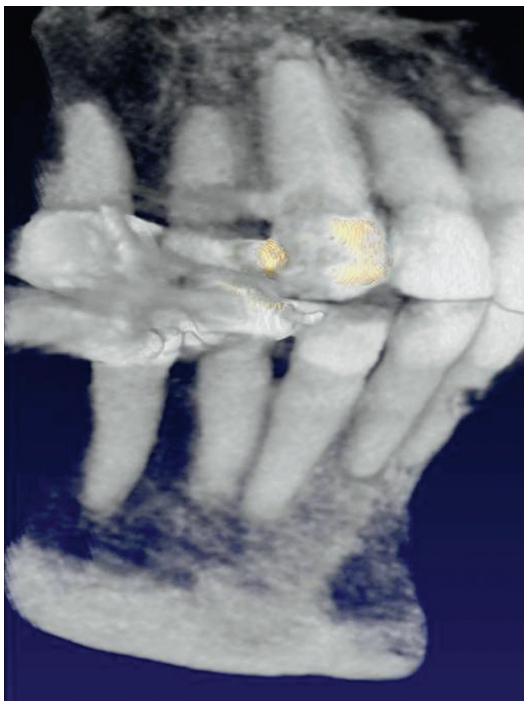
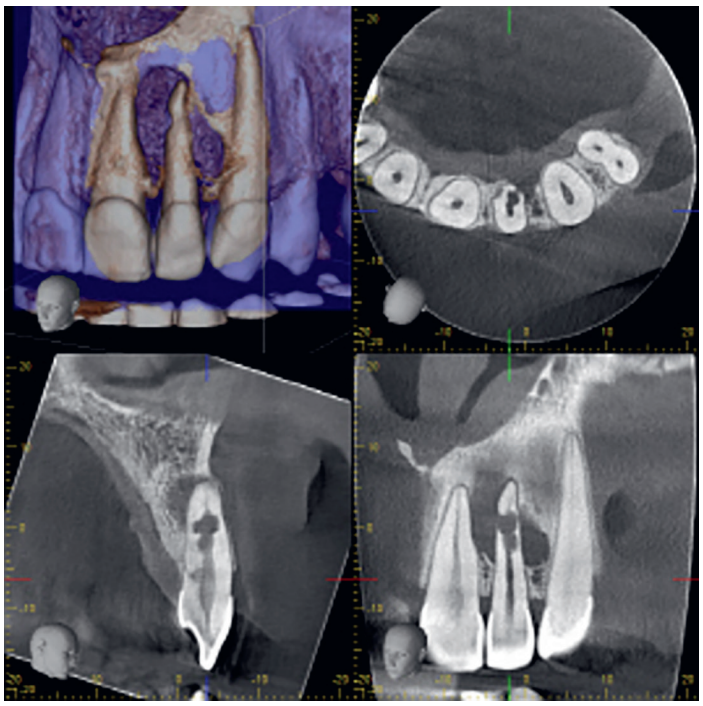


▲ **FIG 13-27** Proximal volumetric reconstruction shows the presence of root resorption on the facial surface of a mandibular right central incisor. (Reprinted with permission from Cohenca et al 2007.)



▲ **FIG 13-28** Internal root resorption (apical) that has perforated; the adjacent bone is resorbed. Perforation is apparent on the sagittal and axial views.

distinguish whether the external root defect is buccal or palatolingual by using the buccal object rule. In internal resorption, the outline of the root canal is usually distorted, and the root canal and the radiolucent resorptive defect appear contiguous. When the defect is external, the root canal outline appears normal and can usually be seen "running through" the radiolucent defect.

Although intraoral radiography provides an acceptable level of accuracy in the diagnosis of root resorption, the use of CBCT enables 3D imaging of root resorption defects from the coronal, sagittal, and axial views and has proven to be superior to 2D imaging (Patel et al 2009a) (see Fig 13-20). In addition, the associated use of volumetric construction enhances visualization of the portals of entry in the case of external resorption as well as the location and extent of the resorption defect and the degree of osseous involvement (Cohenca et al 2007, Patel and Pitt Ford, Patel et al 2009a, Patel and Beddis) (Fig 13-27). Improvements in diagnostic accuracy with the use of CBCT also increase the likelihood of correct management of resorptive lesions (Cotton et al).

External inflammatory root resorption is always accompanied by resorption of the bone in addition to the root (see Fig 13-12). Therefore, radiolucencies are apparent in the root as well as the adjacent bone. Internal root resorption does not involve the bone; as a rule, the radiolucency is confined to the root. On rare occasions, if the internal defect perforates the root, the adjacent bone is resorbed and appears radiolucent on the radiograph (Fig 13-28).

## Diagnostic testing

External inflammatory resorption on the apical and lateral aspects of the root involves an infected pulp space, indicated by a negative response to sensitivity tests. On the other hand, because ICR does not involve the pulp until it has advanced into the root canal, this type of resorption is often associated with a normal response to sensitivity testing. Internal root resorption usually occurs in teeth with vital pulps and elicits a positive response to sensitivity testing. However, teeth that exhibit internal root resorption can sometimes register a negative response to sensitivity testing. This is seen in cases where the coronal pulp is removed (pulpotomy) or necrotic and the active resorbing cells are located more apically in the canal. In addition, the pulp may become necrotic after active resorption has taken place and the appearance of the resorptive defect is archival.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### Pink spot

Because the pulp is nonvital in both apical and lateral external root resorption, the granulation tissue that produces a pink spot is not present in such cases. However, a pink spot caused by granulation tissue that undermines enamel can be a sign of both ICR and internal root resorption (see Fig 13-18).

### Common misdiagnoses

The majority of misdiagnoses of resorptive defects are made in distinguishing between ICR and internal root resorption. As previously mentioned, CBCT is invaluable in differentiating between the two entities. There are clinical signs as well. When root canal therapy is the treatment of choice for an apparent internal root resorption that has not perforated the periodontium, bleeding within the canal should cease quickly after pulp extirpation if the blood supply to the granulation tissue is from the apical blood vessels. However, if bleeding continues during treatment, particularly if it is still present at the second visit, the source of the blood supply is external, and treatment for external resorption should be initiated. Also, upon obturation in cases of internal resorption, it should be possible to fill the entire canal from within. Failure to fill the canal suggests an external communication. Finally, if the blood supply of an internal resorption defect is removed upon pulp extirpation, any continuation of the resorptive process on posttreatment evaluation radiographs should alert the dentist to the possibility that an external resorptive defect was misdiagnosed.

## Systemic Causes of Root Resorption

The roots of teeth show a remarkable resistance to detectable resorption, even with systemic diseases that can cause significant bone resorption. With hyperparathyroidism osteitis deformans (Paget disease), for example, radiographically apparent bone resorption is not accompanied by resorption of the roots (Smith and Eveson). However, hormonal disturbances and genetic factors have been shown to sometimes cause resorption of the roots (Pizzo et al, Hartsfield et al, Sharawy et al, Kuperstein et al). Systemic diseases that have been associated with root resorption include hypophosphatemia, hyperparathyroidism, bone dysplasia, Papillon-Lefèvre syndrome, renal disease, liver disease, and Ehlers-Danlos syndrome type VIII (Carter et al, Gold and Hasselgren, Moskow). While osteoporosis is not directly related to root resorption, the rebound effect of stopping certain osteoporotic medications has been shown to result in multiple ICR lesions (Yao et al).

The preponderance of research on the genetic propensity to develop root resorption has been conducted on populations of orthodontic patients. An IL-1β polymorphism has been positively correlated with an increased risk for external apical root resorption secondary to orthodontic tooth movement. Individuals homozygous for the *IL1B* (+3953) allele 1 have a 5.6-fold greater risk of root resorption than do control populations (Hartsfield, Al-Qawasmi et al 2003). Whether this same genetic variation plays a role in other types of dental resorption is not presently known. However, genetic linkage is implicated in ICR, as idiopathic external root resorption has been observed in members of the same family (Hartsfield et al). As knowledge and test procedures advance, resorption presently diagnosed as idiopathic will likely be increasingly found to be of systemic or genetic origin with a significant environmental influence.

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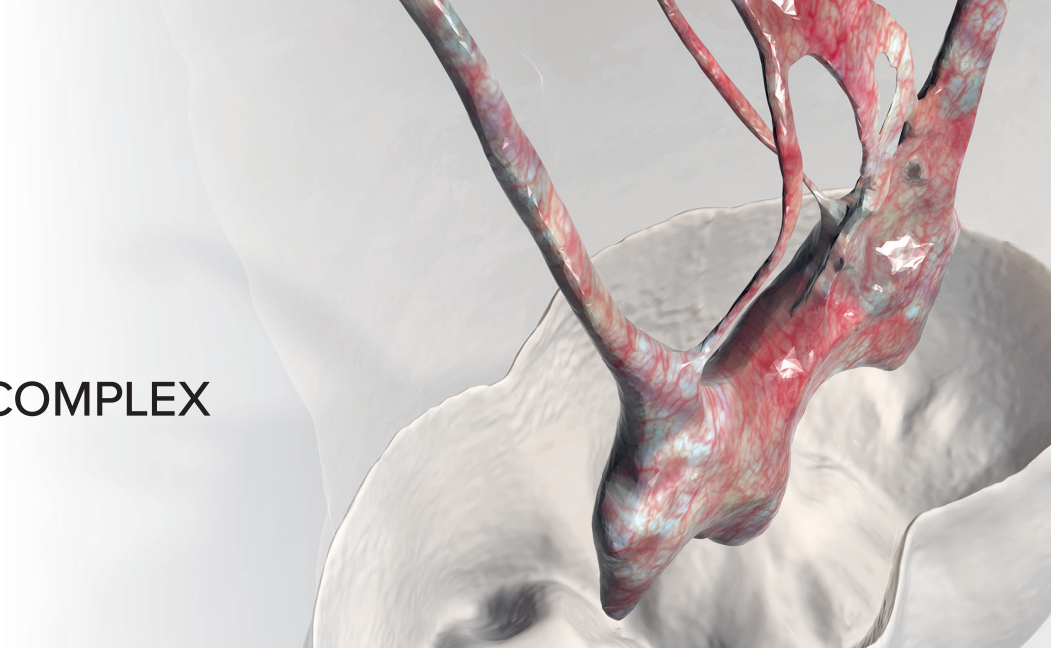
# 14

# AGING OF THE DENTIN-PULP COMPLEX

Scott L. Doyle



Advancements in living standards and health care have contributed to longer life expectancy and a rapidly growing population of older adults worldwide. Over the next three decades, the global number of persons aged 65 years or older is projected to more than double, reaching over 1.5 billion in 2050. The share of the global population aged 65 years or older is expected to increase from 9.3% to 16.0% by 2050 (United Nations Department of Economic and Social Affairs, Calabrese and Rawal).

Today, one in six Americans is over 65 years old (US Census Bureau). By 2030, one in five Americans, or around 70 million individuals, will be 65 years or older, and by 2060, that number will rise to 98.2 million (Colby and Ortman). The population of older adults will be diverse with respect to race/ethnicity, socioeconomic level, health, and functional status (Mather et al).

Despite improvements in health care, the expanding population of older individuals remains at risk for chronic conditions, including oral disease (GBD 2017 Oral Disorders Collaborators). Dental caries, including root caries, is one of the most prevalent conditions among the older adult population (Badr and Sabbah, AlQranei et al, Bashir). Moreover, nearly two-thirds of American adults aged 65 years or older have periodontal disease (Eke et al).

Oral health is an essential component of overall health. It influences the physical, mental, and social well-being of a person and significantly impacts quality of life (Jiang et al). There is an overwhelming body of evidence showing that retaining a healthy natural dentition in older adults is structurally, functionally, and psychosocially beneficial (Müller et al, Iwasaki and Hirano). A recent systematic review concluded that having a functional dentition was positively associated with an increased oral health–related quality of life (OHRQoL) in adults 65 years and older (van de Rijt et al).

The improvement in oral health is attributable to a greater emphasis on prevention and advancements in treating and preserving teeth. Due to a variety of factors, older adults are retaining more of their natural teeth (Griffin et al, Thompson and Chen). With an increased emphasis on tooth retention and a high prevalence of apical periodontitis, many older adults are seeking endodontic care (Hamedy et al). Endodontists have reported an increased number of patients aged 65 years or older in their practices, and this is expected to increase in the future (Goodis et al).

The increased need for endodontic treatment among older individuals is due to naturally occurring anatomical and physiologic senescent changes that are associated with the aging process, as well as pathologic changes that occur more commonly in older adults. This chapter reviews age-related processes in the dental pulp, dentin, cementum, and associated structures. Understanding these changes should aid in the management of an increasingly older adult population.

## Age-Related Changes in the Pulp

The dental pulp, like other body tissues, undergoes age-related alterations that are difficult to differentiate from physiologic, protective, and pathologic changes (Quigley). These changes have largely been observed in animal experiments and human studies through examination of extirpated dental pulp tissue or tissue harvested from extracted teeth.

Age-related morphologic, histologic, and functional changes occur in the dentin-pulp complex (Johnstone and Parashos, Carvalho and Lussi, Maeda). The dentinal thickness of the tooth increases with age, resulting in a

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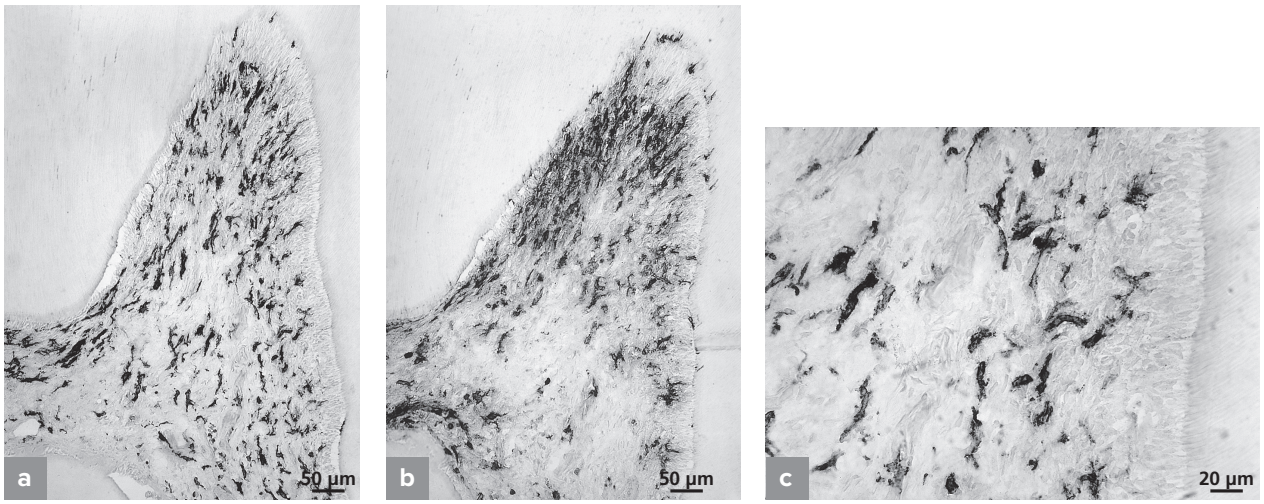


▲ **FIG 14-1** *(a to c)* ED2-positive cells in the coronal pulp of the mandibular first molar of a 24-week-old rat. Positively stained cells are predominantly irregular or dendritic (OX6 stain; original magnification ×130). (Reprinted with permission from Okiji et al.)

progressively more restricted pulp chamber and root canal system (Burke and Samarawickrama). This is the result of continuous physiologic secondary dentinogenesis, as well as tertiary dentin formation (Tziafas 2010). One study demonstrated that dentinal thickness increases by a total mean of 20 µm per year (10 µm per dentinal wall) in the root and increases by a mean of 13 µm per year (6.5 µm per dentinal wall) in the crown (Murray et al 2002).

In general, there is a decrease in the quantity and quality of cells present (Daud et al). Changes in the number, structure, and quality of blood vessels, nerve fibers, connective tissue, and other components of the pulp also occur with age (Morse, Bernick and Nedelman, Ketterl). Consequently, the pulp becomes less cellular and more fibrous, and its nerve and blood supply decrease with age (Yu and Abbott 2007).

## Histologic and histomorphologic analyses

### Animal studies

Several well-controlled animal studies have focused on age-related changes in cell proliferation and collagen turnover. Early studies on the cell kinetics of aging in rat molar pulp cells found detectable DNA synthesis up to 400 days of age, indicative of continual cell division and cell turnover in older animals. The proliferative activity of the pulp was greater in younger rats, while the volume of pulp tissue was reduced by continual dentin apposition in aging animals (Pinzon et al). Using stains and silver impregnation for collagen and reticular fibers, histologic studies found changes in connective tissue collagen (Hayakawa et al).

Examination of the cellular dynamics of the incisor pulps of different-aged rats showed no consistent change in odontoblast number with advancing age. However, a slight but continuing reduction of basal pulp cells occurred throughout life (Lavelle). Thymidine-labeling studies indicate that proliferative activity of fibroblasts contained in the pulp and periodontal ligament is reduced with age (Toto et al). The pattern of decreased activity seems to follow that of human pulp, with DNA synthesis showing a significant decrease over time. Increases in fibrosis may be due to increases in the age-related accumulation of insoluble collagen that accompanies decreased cellularity. In bovine studies, collagen concentrations increased from about 9% to 25% as pulp matured, and the ratio of type 3 to type 1 collagen also increased, suggesting a substantial increase in type 3 synthesis with age (Moxham et al, Webb et al). There are also age-related alterations in the levels and distributions of the cytoskeletal proteins actin, cytokeratin, and vimentin. The levels of these proteins diminish with age, and the decreases are most striking in odontoblasts (Moxham et al, Webb et al).

Animal studies have also found age-related changes in the immune system in dental pulp. Examination of immunoglobulins in bovine dental pulp found that immunoglobulin G levels decreased as the animal aged (Fishman et al). Alterations in the levels of immunoglobulins may indicate maturation of the animals and may reflect physiologic changes rather than the presence of localized pathoses. This finding is consistent with other evidence that the overall immunodefense potential of the pulp decreases in older animals (Okiji et al) (Figs 14-1 and 14-2). The distribution of pulp cells expressing reactivity to monoclonal antibodies was examined immunohistochemically in the mandibular first molars of developing (newborn to 10-week-old), adult (14- to 24-week-old), and aged (1- to 15-year-old) Wistar rats. Results indicated


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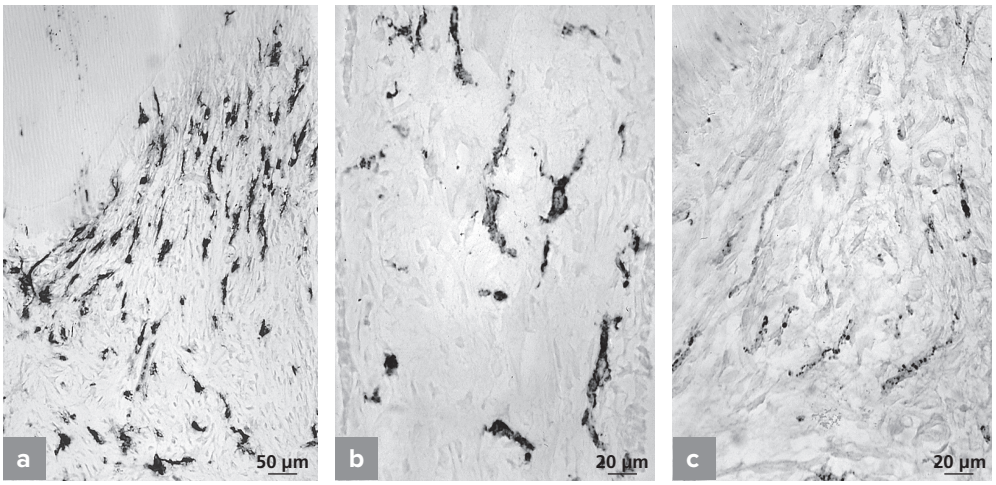


▶ **FIG 14-2** *(a to c)* Distribution of macrophages in the coronal pulp of the mandibular first molar of a 36-month-old rat. Positively stained cells are predominantly elongated (OX6 stain; original magnification ×130). (Reprinted with permission from Okiji et al.)

that monoclonal antibody–labeled cells were maintained throughout life, but a significant decrease of OX6-positive (Class II major histocompatibility complex–expressing) cells was observed. The density and type of pulp cells expressing macrophage-associated antigens reduced with advancing age, most likely due to alterations in the pulp's immunologic defense against infection (Okiji et al).

A later study used rats in two age groups. The dental pulp monocyte/macrophage system response to cavity preparation in animals 12 and 18 months old was compared to that in younger animals 3 and 6 months old. No differences were found in the monocyte/macrophage defense mechanism to injury in either group, indicating the possibility that the dental pulp retains the ability to defend itself against injury during the aging process (Kawagishi et al).

### Human studies

Histologic analysis of human teeth confirms that with age comes a reduction in cell numbers and a narrowing of the pulpal space (Elfenbaum). Similarly, when the cellularity of the outer cell-rich zone of pulp was compared to the less cellular middle zone, it was found that the cell number decreases in all three zones starting at 20 years of age; by the age of 70 years, the total cell number is reduced by 50% (Fröhlich).

Murray et al (2002) confirmed and quantified more precisely the reduction in cell density and compared the pulp in the crown with the pulp in the root. In comparison with younger patients aged 10 to 30 years, the number of crown odontoblasts decreased by 15.6% for all teeth and the density of root odontoblasts decreased by 40.6% in patients 50 to 59 years old. Similarly, the density of fibroblasts decreased by 26.9% in the crown and by 41.3% in the root with aging (Murray et al 2002).

A similar study reinforced that the density of odontoblasts, subodontoblasts, and fibroblasts was higher in the coronal portion of the pulp than in the root. In general, the pulp cell density decreased with age in both the crown and root regions. However, coronal odontoblast decrease occurred later in life (40 to 49 years) compared to subodontoblasts and fibroblasts (30 to 39 years). Histomorphologic examination of the older cells revealed that odontoblasts were more closely arranged, ovoid in shape, and shorter in length. Subodontoblasts became progressively flattened, with scant cytoplasm. Fibroblasts appeared flattened and spindle-shaped (Daud et al).

Histologic analysis of 100 noncarious molars from males and females aged 15 to 75 years found that aging was associated with increased calcification of the extracellular matrix, decreased numbers of blood vessels and nerves, increased fibrosis (collagen sheaths), increased collagen fiber thickness, changes in the chemical properties of collagen fibers, and fusion of von Korff fibers in the odontoblastic area. The prominence of collagen fiber bundles was attributed to the persistence of the connective tissue sheaths in the narrowed pulp chamber after loss of the vascular and nerve supply and not to continued formation and reorientation of collagen fibers during the aging process (Bernick and Nedelman). These findings are consistent with the conclusions of other investigators (Stanley and Ranney).

Fibroblasts are the most abundant cells in the dental pulp (Jeanneau et al). It has been believed that the primary role of fibroblasts is the production and replacement of collagen and other components of the extracellular matrix; however, fibroblasts reportedly have an important role in pulp function. Investigations show that fibroblasts synthesize all complement system proteins, indicating

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their involvement in defensive processes, inflammation regulation, and dentin-pulp regeneration. These cells also produce proinflammatory cytokines and chemokines, express pattern-recognition receptors (which are involved in numerous immunoregulatory mechanisms), and express a number of neuropeptides and their receptors, thereby playing an essential role in neurogenic inflammation (Álvarez-Vásquez and Castañeda-Alvarado). A progressive decrease in number and altered morphology of fibroblasts are part of the pulp aging process.

Clinical studies have also documented age-related changes in collagen in the dental pulp. In human third molar pulps from patients aged 16 to 40 years, there was a substantial increase in calcium and a decrease in levels of dihydroxylysinonorleucine (Nielsen et al). This modified amino acid is a major cross-linker that binds collagen fibers together and can be detected by the addition of reducing agents. Because collagen maturation is characterized by the presence of reducible cross-links, this study demonstrated that collagen synthesis in the coronal pulp decreased with age. This change was accompanied by a decline in collagen concentration in terms of dry weight and total protein.

Another study evaluated 239 noncarious human teeth in individuals from 10 to 78 years of age (Domine and Holz). The older dental pulps were characterized by a relative compression of collagen fibers that resulted from accumulated dystrophic or degenerative calcifications, giving an appearance of fibrosis. Although increased fibrosis was not actually observed in this study, others have reported increased fibrosis and calcification radicularly that is more severe than that found coronally (Badillo and Brouillet). The larger collagen fiber bundles in the mature pulp are more numerous in the radicular pulp, with the highest concentration found at the apex.

The matrix components of 332 human teeth in three age groups (10 to 30 years, 31 to 51 years, and 52 to 72 years) were evaluated (Hillmann and Geurtsen). The results indicated that collagen types 1, 2, 5, and 6 are present at all ages. Staining of type 1 collagen is weak, and staining of types 3, 5, and 6 collagen is strong. With advancing age, fiber bundles of type 1 collagen increase in frequency and thickness while the fine fibrils of types 3, 5, and 6 collagen disappear or are replaced by thick fiber bundles. The connective tissue matrix appears condensed and stained homogenously in older individuals.

Transcriptome analysis was conducted to clarify the genetic changes that underlie the histologic modifications in the dental pulp with aging (Tranasi et al). Microarray analysis of third molars of 18- to 20-year-old and 57- to 60-year-old patients revealed several differentially expressed genes that were categorized as encoding growth factors, transcriptional regulators, apoptosis regulators, and components of the extracellular matrix. In young dental pulp, high expression levels were detected for genes involved in cell proliferation, cell and tissue differentiation, and cell development as well as in the immune, lymphatic, and hematologic systems; in older dental pulp, genes involved in apoptosis were more highly expressed. Higher expression of genes for synthesis products (collagenous and noncollagenous) was noticeable in the younger dental pulp, whereas genes encoding specific markers such as dentin sialophosphoprotein, ameloblastin, or osteonectin were highly upregulated in the older dental pulp. Although proteomic experiments are needed to confirm the differential gene expression at the protein level, these results are interesting because they may be related to the intrapulpal calcification and mineralization observed during aging.

Osteocalcin is a vitamin K–dependent, noncollagenous dentin and bone matrix protein that is generated by osteoblasts and odontoblasts. It is a well-known marker of cell viability, differentiation, and osteogenic potential in these cells. It has been postulated to inhibit excessive mineralization (Muramatsu et al 2005). One study compared third molar pulps of 17- to 25-year-olds with that of patients aged 50 years or older (Ranly et al). Using osteocalcin as an index of matrix production, the researchers found that osteocalcin expression (presumably of odontoblast cell lineage) does not diminish relative to the explant cell population. The authors concluded that despite a reduction in tissue volume and cell numbers, the pulps of aging teeth retain a capacity for dentin deposition. Using different methodology, another study showed decreased osteocalcin mRNA expression in aged human dental pulp, suggesting that this change may be associated with loss of viability and may be a characteristic of aging (Muramatsu et al 2005).

A higher level of autophagy has been suggested to occur both in aged human pulp tissues and in cultured senescent dental pulp cells (Lee et al, Li et al). This highlights a unique role for autophagy in aging dental pulp cells. It has been found that lipopolysaccharide (LPS) stimulation induces a different autophagy activity in young and aged dental pulp cells. Upon LPS stimulation, the level of autophagy declines only in young cells but is retained in aged cells (Lee et al). This has been associated with aging-related decline of peroxisome proliferator-activated receptor-γ in aging dental pulp cells (Yang et al).

Alkaline phosphatase (ALP) is an enzyme that is involved in the induction of mineralization in the dental pulp. Aged human dental pulp cells may show reductions in proliferation and ALP activity. The in vitro proliferative life span of human pulp cells from young donors was longer than that of cells from older donors. Growth rates and ALP activity decreased with increasing donor age.


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These findings suggest that impaired repair of pulp and dentin in aged patients is partly due to a decrease in the proliferative ability and ALP activity in aged pulp cells (Shiba et al). Another study investigated and compared the ALP levels in healthy and inflamed pulp tissue samples from young (< 30 years old) and old (> 60 years old) donors (Aslantas et al 2016). Healthy pulp samples were obtained from extracted third molars. For reversible and irreversible pulpitis, pulp samples were obtained during endodontic treatment. ALP activity was assessed by spectrophotometry and immunohistochemistry. In cases of low-grade inflammation (hyperemia), both young and old pulp samples had a slight increase in ALP activity. In cases of irreversible pulpitis, ALP enzyme activity was significantly increased in older individuals. The authors suggest that in aging individuals, increased severity of inflammation may trigger calcification. It was concluded that an increase might represent different age-dependent mineralization and inflammatory reactions rather than acting as an indicator of viability or protective effect of pulp cells (Aslantas et al 2016). Therefore, assessment of dental pulp status in the aging process may also help to determine the defensive capacity of pulp cells.

Catalase is an antioxidant enzyme that catalyzes the conversion of hydrogen peroxide to water and molecular oxygen. A recent study examined the effects of aging on catalase activity in human dental pulps (Aslantas et al 2022). Sixty samples from young (< 30 years) and old (> 60 years) donors were used for spectrophotometric analysis and immunohistochemical evaluation. Healthy pulps were obtained from extracted third molars. Inflamed (reversible and irreversible pulpitis) samples were obtained by pulp extirpation during endodontic treatment. Catalase enzyme activity was determined by spectrophotometry, and fixed tissue was evaluated for catalase immunoreactivity (Aslantas et al 2022). Catalase enzyme activity in healthy pulp samples of older participants was significantly higher than in healthy samples of younger participants. In addition, catalase activity in samples of reversible and irreversible pulpitis was considerably lower in the older group compared to healthy control samples. In contrast, enzyme activity was considerably higher in reversible pulpitis samples from younger participants than in irreversible pulpitis samples and reversible pulpitis samples from older participants. Immunohistochemical evaluation indicated that there was intense catalase immunoreactivity in young patients with reversible pulpitis compared with reversible pulpitis in the older group. However, weak immunoreactivity was observed in samples from young participants with irreversible pulpitis and old participants with reversible pulpitis. The authors concluded that pulp tissue presented different levels of catalase activity against pulpitis and aging. The findings of this study suggest that inflammation produces different signaling cascades in young and old pulp cells (Aslantas et al 2022).

## Odontoblasts

Odontoblasts are long-lived postmitotic cells organized in a continuous palisade at the dentin-pulp interface, where they maintain predentin and dentin apposition for the entire life of a healthy tooth. The odontoblastic layer undergoes a progressive reduction in thickness with aging, mainly due to a reduction in cell size (Couve et al 2012). Human odontoblasts can be divided into three functional phenotypes: secretory, mature, and old (Couve et al 2013).

The odontoblast retains some secretory and sensory capacity until cell death. Under severe injury, such as rapidly progressing caries, damaged odontoblasts are replaced by odontoblast-like cells from the subodontoblastic layer, which can form reparative tertiary dentin (Smith et al, Tziafas 2019, Kawashima and Okiji).

The longevity of odontoblasts is sustained by an elaborate autophagic-lysosomal system that ensures the turnover and degradation of cellular components (Yang et al). However, this pathway has been shown to be diminished in aging odontoblasts. The content, size, and location of autophagic vacuoles change with age. Progressive accumulation of undegradable lipofuscin deposits within lysosomes indicates reduced lysosomal activity as a function of odontoblast aging (Couve and Schmachtenberg 2011). Additionally, mitochondrial elements converge around large clusters of lipofuscin deposits. The progressive accumulation of intralysosomal debris limits the autophagic turnover of mitochondrial components. Autophagy of damaged mitochondria, known as *mitophagy*, is reduced in aged odontoblasts (Couve et al 2012).

Although the mature odontoblast stage might remain for decades, aging effects and subtle age-related alterations become apparent over time (Figs 14-3 and 14-4). Reduction in autophagic activity and the progressive accumulation of lipofuscin characterize an "old" odontoblast condition in adult patients, represented by a reduced cell size and flattened shape (Couve et al 2012). In response to stress, odontoblasts' survival and functional viability decline as they age (Couve et al 2013). In addition, clinical studies have shown that the dentinogenic response of odontoblasts to mechanical injury decreases with age (Murray et al 2000, Murray et al 2003).

Gap junctions between human odontoblasts are mainly formed by connexin 43 (Cx43). Reduced expression of this protein has been demonstrated in aged pulps. This may be associated with the loss of viability in human dental pulp and is considered one characteristic of aged pulp (Muramatsu et al 2004).

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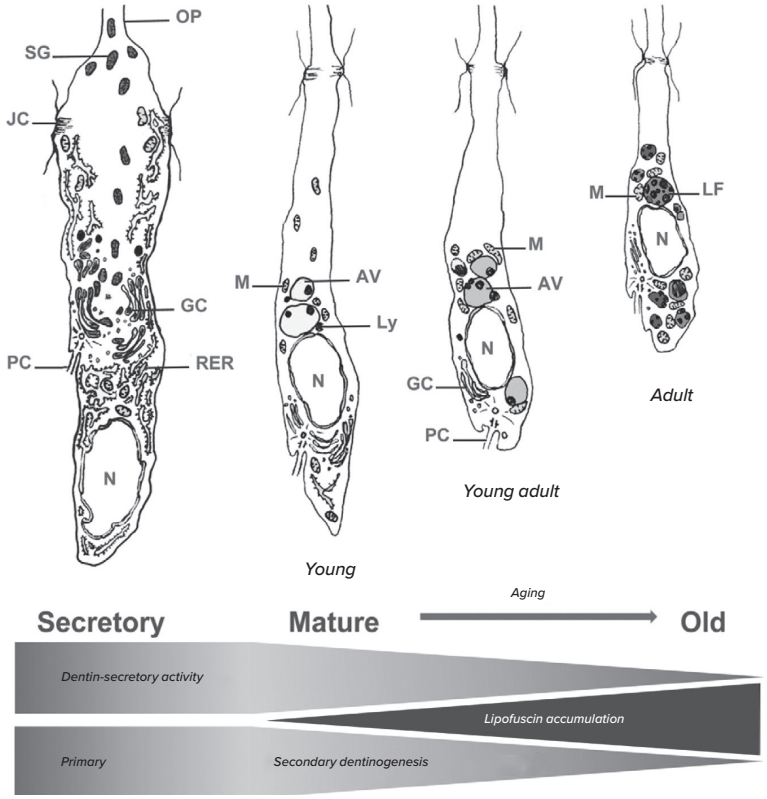
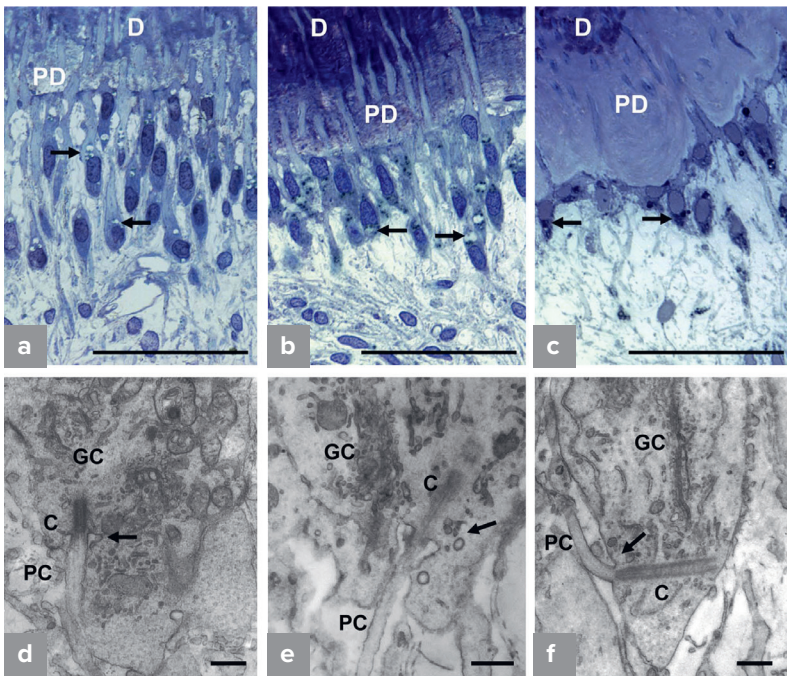



◀ **FIG 14-3** Schematic representation of the life cycle of human odontoblasts. During tooth development, secretory odontoblasts are the primary dentin-forming cells. After tooth eruption of the coronal dental pulp, odontoblasts reduce their secretory machinery and enter a mature stage characterized by the presence of autophagic vacuoles (AV). The progress of aging in mature odontoblasts involves the accumulation of lipofuscin deposits (LF). Three individual age groups are denoted below their corresponding odontoblast condition. GC = Golgi complex; JC = junctional epithelium; Ly = lysosome; M = mitochondria; N = nucleus; OP = odontoblastic process; PC = primary cilium; RER = rough endoplasmic reticulum; SG = secretory granules. (Modified with permission from Couve and Schmachtenberg 2011.)




◀ **FIG 14-4** Mature human odontoblasts. *(a to c)* Light microscopy of the coronal odontoblastic layer from young (15 y), young adult (25 y), and adult (75 y) teeth. Odontoblasts in young *(a)* and young adult *(b)* individuals are columnar cells with large autophagic vacuoles *(arrows)*, while in adult individuals, odontoblasts are shorter and flattened cells with dense deposits accumulated within autophagic vacuoles *(c, arrows)*. *(d to f)* High-magnification transmission electron microscopy of the primary cilium (PC). In odontoblasts from young *(d)* and young adult *(e)* individuals (15 and 25 y), the PC has a well-defined ciliary pocket structure with numerous membranous components in close association *(arrows)*. *(f)* PCs are persistent structures in odontoblasts from adult (75 y) individuals. C = centrosome; D = dentin; GC = Golgi complex; PD = predentin. Scale bars = 50 mm *(a to c)* and 0.5 mm *(d to f)*. (Modified with permission from Couve and Schmachtenberg 2011.)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## Blood vessels

The circulatory system of the pulp is extensively reviewed in chapter 3. The dental pulp is richly supplied by a microcirculatory system comprising precapillary arterioles, capillaries, and postcapillary venules (Yu and Abbott 2018). The arterioles enter through the apical foramen in a neurovascular collagen bundle, ascend toward the coronal region (giving off numerous branches en route), and terminate in a rich subodontoblastic capillary plexus (Yu and Abbott 2018). Drainage of blood occurs via venules that exit the tooth through the apical and lateral foramina. There is some evidence for the existence of arteriole-to-arteriole, arteriole-to-vein, and vein-to-vein anastomoses, suggesting that there is extensive regional control of blood flow to the pulp (Takahashi et al).

The microvascular supply to the pulp is small relative to the volume of tissue that it supports. The number of vessels entering the tooth diminishes with age, and the vascular plexus becomes reduced, sometimes entirely absent. Additional age-related changes include intimal hyperplasia of arterioles, narrowed vessel lumens, and calcification of vessel walls (Bernick 1967a). Researchers observed reduced vascularity in coronal pulp with increasing age, although radicular vascularity was observed, indicating the presence of an effective, anabolically active tissue (Domine and Holz).

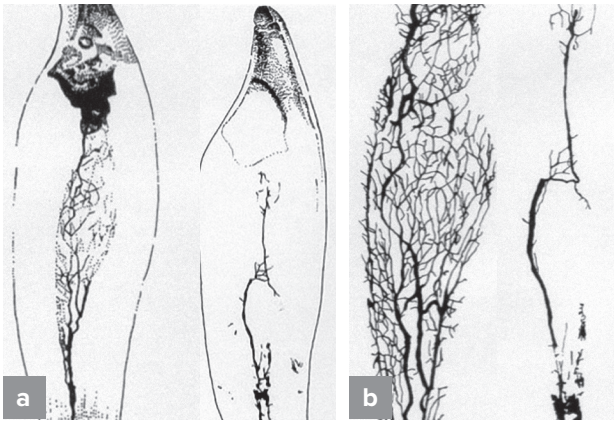
Odontoblast activity and viability are tied closely to the vascular supply, notably the supply from the capillary plexus positioned next to the preodontoblast and predentin layer. With the age-related reduction (compression) of the feeder arteriole system to the pulp, there is an associated diminution of the capillary plexus, including vessel degeneration near the incisal end of the pulp. There is also a loss of fenestration in the capillaries.

It is highly likely that these marked age-related changes in the vascular supply to the pulp contribute to the diminution in the capacity of odontoblasts to survive and produce new dentin matrix. To examine this question, one study of pulp vascularity collected single-rooted, permanent human teeth from individuals in three age groups: 10 to 20, 20 to 40, and 40 to 70 years (Bennett et al). With increasing age, the teeth showed reduced terminal branching of vascular structures and reduced numbers of vessels entering and exiting the apical foramen. Teeth in the 40- to 70-year group demonstrated the fewest number of vessels, often having only one primary arterial vessel entering the tooth compared to three or four vessels in the 10- to 20-year age group (Fig 14-5). The peripheral capillary plexus was also reduced or entirely absent in most pulps of the older age group.

A classic study compared teeth from individuals younger than 20 years and individuals aged 40 to 70 years and demonstrated a highly vascularized tissue in the younger group; vessels of arteriole size were found in the coronal portion of the tissue of younger teeth (Bernick 1967a). The pulps from older patients were characterized by a gradual narrowing of the circumference of the pulp and vessels that became more prominent centrally. As secondary dentin formation continued, the pulpal area and circumference decreased, leaving only a thread of pulp tissue and causing narrowing and elimination of medium-sized vessels and capillaries.

A qualitative and quantitative study evaluated changes in ALP and adenosine triphosphatase activity in three groups of patients (10 to 30, 31 to 50, and 51 to 70 years). Results demonstrated decreases in both enzymes with aging (Ma et al). The most notable change in the expression of these phosphatases occurred in the endothelial cells of the capillaries. The study indicated that reduction of these substances would be accompanied by a reduction in metabolic activity of the pulp with aging, which may indicate a finite ability of odontoblasts to form dentin.

The vascular endothelium has a role in nutrient release, metabolite exchange, and removal of waste products. An ultrastructural study compared the changes that occur in the endothelial wall cells of blood capillaries in older (more than 60 years of age) compared to younger pulps (Espina et al). The endothelial cell cytoplasm of older pulp vessels had more pinocytotic vesicles, microvesicles, and microfilaments. Lipid-like vacuoles, monogranular glycogen granules, and extensive Golgi complexes with dilated cisternae were also present. With advanced age, the capillary endothelium undergoes morphologic changes such as increased transendothelial transport, cytoskeletal changes, Golgi complex hypertrophy, and the formation of cytoplasmic deposits.




▲ **FIG 14-5** *(a)* High-magnification view of the central portion of the pulp chamber of an incisor from a 30-year-old patient. The interior vascular structures are reduced in number, and the peripheral arcade is less intense (original magnification ×100). *(b)* High-magnification view of the pulpal structures of an incisor from the older age group (40 to 70 years). Centrally located major vessels are visible, although interrupted, and the subodontoblastic capillary plexus is absent (original magnification ×100). (Reprinted with permission from Bennett et al.)

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A recent study used laser Doppler flowmetry to assess pulpal blood flow in maxillary incisors. Mean pulpal blood flow levels were significantly lower in older adults (60 to 70 years) than in young participants (20 to 25 years). Additionally, wavelet spectral analysis using variable frequencies evaluated vascular control mechanisms. Myogenic activity was significantly higher in older adults, suggesting age-related increase in vascular tone. Sympathetic activity was significantly increased in older adults, suggesting an age-related increase in the contribution of the sympathetic central mechanism in pulpal blood flow regulation. Values corresponding to endothelial activity were significantly lower in older adults, suggesting age-related endothelial dysfunction (Dzeletovic et al 2020b).

The same research group again used laser Doppler flowmetry to evaluate the effect of carotid atherosclerosis on blood flow oscillations. They showed a reduction in pulpal blood flow in the older sample group (Dzeletovic et al 2020a). An earlier study also observed functional age-related changes in pulpal blood flow (Ikawa et al). Alternatively, another study used Doppler ultrasound to assess pulpal blood flow velocity and demonstrated that only systolic blood pressure was associated with flow, while age was not a significant factor (Kim and Park).

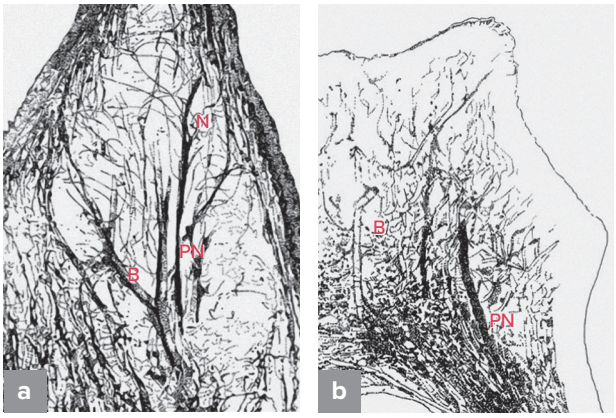
Collectively, these studies of the aging dental pulp demonstrate that vessels decrease in number and that the pulp chamber narrows with deposition of secondary dentin. Even within the context of a smaller tissue volume, a diminished vasculature appears incapable of supplying adequate nutrients to the tissue, resulting in reduced metabolic activity. This combination of senescent changes probably affects the ability of the remaining tissue to react appropriately to an adverse stimulus, resulting in reduced capacity of older individuals and therefore requiring an increased number of restorative or endodontic procedures in this population.

## Sensory nerves

The innervation patterns of pulpal neurons and their responses to injury are described in chapter 4. In general, it should be appreciated that nociceptor responses to tissue injury protect the individual from harm. When that response is diminished or absent, damage to the tissue may occur, necessitating clinical intervention to prevent permanent damage and loss of function.

The nerve supply to the dental pulp is a unique tissue in humans in that its afferent innervation is almost entirely from nociceptors, including Aδ- and C-fibers (Nair, Zhan et al). A reduction in the nerve supply to teeth is well documented to occur with age. This loss appears to parallel the age-related loss observed in vascular supply described earlier. Indeed, the afferent blood supply and the nerves constitute a neurovascular bundle that generally terminates together peripherally.

Histologic examination of pulpal neurons has demonstrated age-related changes (Bernick 1967b). Pulps from 150 teeth in individuals aged 40 to 70 years were sectioned. Mineralization (calcification) occurred in 90% of all pulps. This was first seen in isolated regions in the endoneurium and perineurium of nerve fibers, although older specimens were characterized by mineralization of the entire endoneurial or perineural connective tissue and often appeared to have a calcified ring around the nerve fibers. The younger control group had no calcifications and demonstrated evidence of pulpal nerve branching in the subodontoblastic plexus near the pulp horn region (Fig 14-6a). In the sample of older pulps, there was a decreased overall number of nerves, with few fibers seen in the subodontoblastic plexus area (Fig 14-6b). The nerves that persisted in the pulps of older teeth were often characterized by degenerative changes including fragmentation, beading, and reticulation. This decrease in nerve quality




◀ **FIG 14-6** *(a)* Thick section of the coronal portion of the pulp of a premolar from a teenage individual. There is extensive branching of the pulpal nerves and a rich subodontoblastic network. B = blood vessels; PN = pulpal nerve; N = nerve fascicularis (Verhoeff's iron hematoxylin stain; original magnification ×50). *(b)* Thick section of the coronal portion of the pulp of a molar tooth from a 50-year-old individual. Only the pulpal nerve (PN) is demonstrable; the cuspal branches and subodontoblastic network are absent. B = blood vessels (Verhoeff's iron hematoxylin stain; original magnification ×50). (Reprinted with permission from Bernick 1967b.)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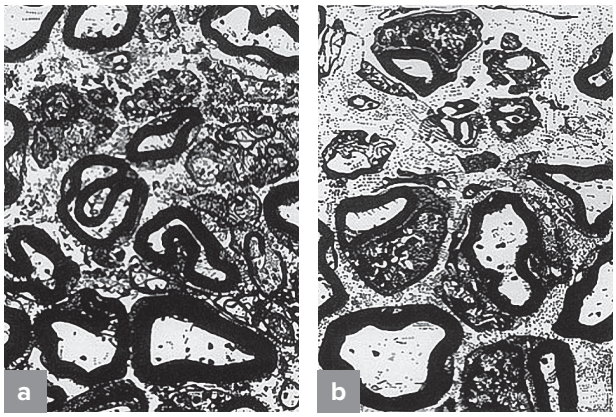
and quantity may explain the reduction in pulpal sensitivity anecdotally reported in aging individuals.

Quantitative assessment of sensory nerve development also demonstrated age-related changes (Johnsen et al, Johnsen). Premolars from patients in four age groups (11 to 15, 20 to 35, 35 to 50, and 50 to 71 years of age) were tested with an electric pulp tester prior to removal. They were then examined for the number of axons present in the apical third of the pulp. The results showed that myelinated axons were greatest in number in patients 15 years of age or older, with a trend to fewer myelinated axons in the older age groups (Fig 14-7). In older teeth, small, unmyelinated axons predominated. Younger teeth, with larger numbers of myelinated and unmyelinated fibers, had a lower threshold of response when tested with an electric pulp tester than did older teeth with fewer fibers. The increased threshold for stimulation in older teeth probably is due to both a loss of the total number of fibers and a reduced terminal arborization of remaining fibers.

Detailed studies of age-related changes in pulpal sensory neurons have been conducted in cats. A study in cat incisors showed an increase in the number of axons in the apical region up to the third year and reduced numbers of myelinated axons from 3 to 11 years of age (Fried 1987). These axons were also smaller in relation to myelin sheath thickness. The inferior alveolar nerve underwent retrograde changes, probably due to pulpal axon degeneration, and the axons appeared to be of a rather transient nature. In a comparison study, the same results were seen in feline primary incisors but with the added aspect of resorption (Fried and Hildebrand). One possible explanation for the pulpal axon degeneration seen in these teeth could be the early disappearance of odontoblasts, as the axons are closely associated with these cells.

In another study, pulps from five healthy cats (3 to 10 years of age) displayed an age-related decrease in the number of pulpal axons, nerve fiber diameters, and intermodal lengths (Fried and Erdélyi). The decrease in intermodal lengths may be due to demyelination and remyelination, as aging fibers exhibited qualitative changes in their myelin sheaths. Age changes in the peripheral nervous system may be related to neuron aging, giving rise to distal axon degeneration, but may also be due, as noted, to age-related changes in the target tissue.

There is an age-related change in the ratio of myelinated to unmyelinated neurons in human dental pulp. One study evaluated the variation in the number of pulpal myelinated nerves in patients aged 10 to 72 years and reported that the total number decreased with age, particularly at the expense of myelinated fibers (Matysiak et al 1986). The decrease in sensory A fibers may be related to reduced sensitivity to the perception of dental pain in aging patients. Another related study measured myelinated and unmyelinated axon populations in the dental pulp and reported that the pulps of older individuals showed a loss of small Aδ-fibers and unmyelinated C-fibers, with a proportionately greater decrease in the number of C-fibers (Matysiak et al 1988).




▲ **FIG 14-7** *(a)* Electron micrograph of a cross section of axons in a premolar from a 15-year-old patient. Unmyelinated axons form groups of varying sizes (original magnification ×6,000). *(b)* Electron micrograph of a cross section of axons in a premolar from a 34-year-old patient. Unmyelinated axons are usually in small groupings (original magnification ×6,000). (Reprinted with permission from Johnsen et al.)

Aging involves both structural and neurochemical regressive changes in pulpal innervation (Fried 1992). Pulpal calcitonin gene-related peptide (CGRP)–, substance P–, and nerve growth factor (NGF) receptor–like immunoreactivity diminished significantly with age. Neuropeptide expression variations are not fully related to nerve fiber loss, because most aging pulps have NGF receptor–positive fibers but lack neuropeptide expression. It is important to understand that these changes appear as a physiologic response to aging.

Comparatively few studies have evaluated age-related decreases in neuropeptide response and modulation of inflammation caused by operative cavity preparations. One study compared pulpal neuropeptide responses to tooth preparations in young (3- to 4-month-old) and old (1- to 2-year-old) rats (Swift and Byers). Examination of the pulp revealed that older animals had a substantial reduction in levels of the low-affinity NGF receptor (p75-NGFR). The authors concluded that this might be a biochemical marker of pulpal aging. After injury, the neuropeptide levels were increased in both young and old rats in pulp adjacent to the Class V preparation site. The older animals displayed a greater proportional neuropeptide response near the site of injury than did the younger animals. This enhanced response could be due to reduced pulpal volume or to lower basal levels. In either case, the results indicated that older rats exhibit appropriate neuropeptide responses to tissue injury despite several age-related tissue changes (Swift and Byers).

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A related study analyzed innervation of pulp in young (6- to 8-week-old and 3-month-old) and older (5- to 12-month-old) mice with a null mutation in the p75 gene and compared the results with age-matched, wild-type controls (Sarram et al). The results showed intense CGRP immunoreactivity in pulpal nerve endings of mutant mice but a gradual decrease in CGRP intensity in controls during aging. Collectively, these studies indicate that there is an age-related reduction in peripheral neurons and neuropeptide expression but that the aged pulp can mount an appropriate neuronal response to tissue injury (Fristad et al).

A more recent study aimed to determine the relationship between changes in the pattern of Schwann cell morphologies between young and aged adult teeth and neuronal, immunologic, and vascular components of the dental pulp (Couve et al 2018). *Schwann cells* are nerve-associated glial cells that support axonal development, maintenance, defense, and regeneration in the peripheral nervous system. Schwann cells form a prominent glial network at the coronal dentin-pulp interface, forming a complex multicellular neuroimmune interface in association with nerve fibers and dendritic cells. Nonmyelinating and myelinating Schwann cell phenotypes are recognized by the expression of specific markers. In young adult teeth, a dense population of nonmyelinating Schwann cells project processes in close association with sensory nerve terminals through the odontoblast layer, reaching the adjacent predentin/dentin. While GAP43 and p75NTR are substantially expressed in nonmyelinating Schwann cells of young adult teeth, their presence decreases significantly in older adult teeth. Myelinated axons, identified by myelin basic protein expression, are mainly present at the Raschkow plexus and within nerve bundles in the dental pulp, but their density is significantly reduced in older adult versus young adult teeth (Couve et al 2018, Couve and Schmachtenberg 2018). These data reveal age-related changes within the glial network of the dental pulp, in association with a reduction of coronal dental pulp innervation in older adult versus young adult teeth (Couve et al 2018, Couve and Schmachtenberg 2018). This study supports previous findings demonstrating a reduction in the sensory and regenerative capacity of the dental pulp with age. This may explain the progression of pulpitis without symptoms in older patients (Michaelson and Holland).

Collectively, these observations show a clear loss of pulpal nerve supply with advancing age. These changes may result from some underlying mechanism of aging that acts on neurons in a fundamental manner. More likely, however, they result from a combination of age-related events including compression (as the pulp space becomes smaller due to secondary dentin formation), fibrosis and calcification, loss of blood supply, and diminution in the fibroblast and odontoblast population (sources of neurotrophic factors).

## Pulp testing

Pulp testing is a critical component in endodontic diagnosis. Laser Doppler flowmetry and pulse oximetry are the most accurate methods to assess vitality of the pulp (Mainkar and Kim). In clinical practice, pulp sensibility tests are more practical. Clinicians utilize these tests to indirectly evaluate pulp status by activating pulpal neurons with electric current and thermal stimulation using cold and heat (Chen and Abbott).

Physiologic and pathologic alterations in the dentin-pulp complex related to age or progressive disease can affect the reliability of sensibility tests. Older patients have a generalized reduction in primary afferent nerve fibers, which may lead to weaker and delayed responses to thermal stimuli (Ikeda and Suda). A decreased pulp chamber volume due to secondary/tertiary dentin deposition and progressive mineralization of the dentinal tubules has also been associated with a reduction in thermal sensitivity in older patients (Carvalho and Lussi).

A clinical study assessed the influence of age on response to cold sensibility tests. Refrigerant spray was applied to 50 patients over 60 years old and to 50 patients aged 20 to 30 years. The older patient group had longer response times and a decrease in pain intensity, suggesting an increase in pain threshold (Farac et al). Another clinical study demonstrated that cold testing was less accurate in patients over 50 years compared to those in the 25- to 50-year age group (Jespersen et al).

Due to the limitations of sensibility tests, investigators have advocated using multiple modalities. A combination of thermal and electric tests may provide the most accurate results in older patients (Alghaithy and Qualtrough, Barczak et al). Other factors to consider when interpreting test results include the restorative status of the tested tooth and medical condition of the patient, as well as the patient's cognitive function and ability to communicate subjective responses (Hazard et al, Kermani et al).

## Pulp stones

Pulp stones (denticles) are mineralized masses of variable size located in the pulp space, usually in the coronal region (Fig 14-8). These can be differentiated from more diffuse pulp mineralization such as dystrophic calcification. Pulp stones can be categorized as free, adherent, or embedded, according to their location in relation to the pulp space walls (Moss-Salentijn and Hendricks-Klyvert). Pulp stones have also historically been classified as "true" or "false" based on composition and morphology (Goga et al).

Many theories have been proposed to explain pulp stone development, but the exact mechanism is not fully understood. Local and systemic factors may influence formation, but age as a direct cause has not been confirmed


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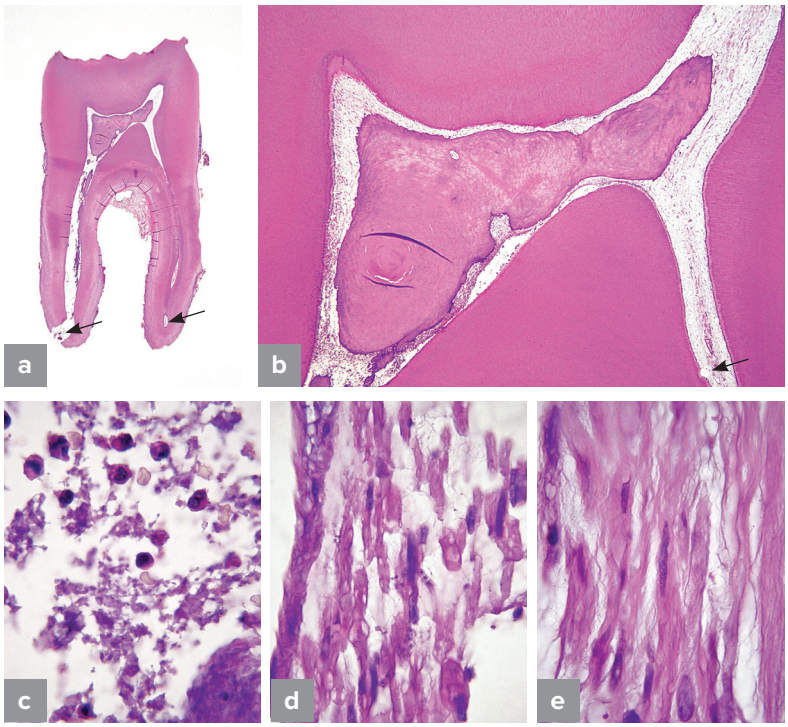


▶ **FIG 14-8** *(a and b)* A large pulp stone occupies the majority of the pulp chamber (hematoxylin-eosin [h&e] stain; original magnification ×6 and ×25, respectively). *(c)* High magnification (×1,000) of the apical foramen of the distal root indicated by the *left arrow* in *a*. Note the necrotic tissue and few polymorphonuclear neutrophils. *(d)* High magnification (×1,000) of the tissue present in the apical portion of the mesial root indicated by the *right arrow* in *a*. Note the nerve fibers and absence of inflammation. *(e)* Coronal third of the mesial root. High magnification (×1,000) of the area indicated by the arrow in *b*. The pulp tissue is free from inflammation. (Reprinted with permission from Ricucci and Siqueira.)

(Sener et al). The prevalence of pulp stones has been evaluated using various geographic samples, histologic criteria, and radiographic modalities. A recent systematic review included 16 studies and 14,093 patients. Meta-analysis revealed a pooled prevalence of 36.53% in the total sample population: 32.58% for men and 39.23% for women. In addition, of 193,687 teeth investigated during the primary studies, 9.57% were affected by pulp stones (Jannati et al).

The morphologic, structural, and chemical properties of pulp stones have been analyzed. The pulp stones revealed a heterogeneous morphology and structure compared with each other. Pulp stones had a similar structure to adjacent dentin. Chemical analysis revealed that oxygen, calcium, carbon, and phosphorus were the most prevalent chemical elements in the inner part of the stones. Carbon, nitrogen, sulfur, chlorine, aluminum, potassium, zinc, copper, and lead were the most prevalent on the surface. Copper, iron, and zinc were higher in the stones than in the dentin (Milcent et al). An earlier study showed similar characterization of the physiochemical properties of pulp stones and dentin (Berès et al).

Pulp stones have been suggested as a diagnostic marker for certain systemic diseases, potentially related to an imbalance in calcium metabolism. A recent systematic review and meta-analysis including 19 studies revealed a significant association between pulp stones and cardiovascular disease but no association between pulp stones and kidney stones. The results also revealed an association between pulp stones and cardiovascular disease in patients older than 40 years (Almadhoon et al). Another systematic review and meta-analysis using two studies revealed an association between pulp stones and kidney stones (Gabardo et al).

The presence of pulp stones is not likely associated with symptoms. Pulp stones may complicate access to canal systems, but magnification and ultrasonics should aid in their identification and removal. Clinicians should be careful to avoid dislodgement of small stones or fragments into the canal space.

## Age-Related Changes in Dentin

There has been a significant amount of research conducted on age-related changes in the microstructure and mechanical properties of dentin. Arola and collaborators have contributed significantly to this increased knowledge base. Many excellent reviews address this topic (Kinney et al 2003, Arola et al 2012, Arola et al 2017).

Dentin is a mineralized tissue that consists of approximately 70% mineral, 20% organic matter, and 10% water by weight (Tjäderhane et al). Dentin forms the bulk of the tooth and provides a resilient supporting foundation for enamel. The organic component is primarily fibrillar type 1 collagen reinforced with apatite minerals. The microstructure

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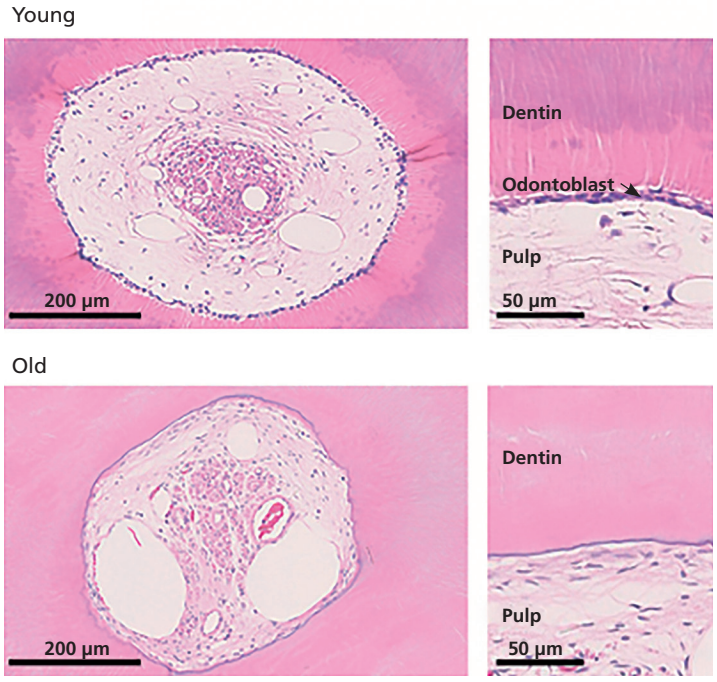
of dentin is largely dominated by its tubules, which extend radially outward from the pulp toward the dentinoenamel junction and cementum. Peritubular dentin is a highly mineralized cuff of apatite crystals that lines the tubules. This tissue is characterized by the absence of type 1 collagen and its high mineral content (Xu and Wang). Intertubular dentin is located between the tubules and is composed of a collagen fibril mesh that is oriented approximately perpendicular to the tubules and is bound by apatite crystallites (Kinney et al 2003, Marshall et al). The collagen fibrils have a diameter of 50 to 100 nm, whereas the apatite crystals are approximately 5 nm thick, and their remaining dimensions are dependent on distance from the pulp (Kinney et al 2001, Arola et al 2012). Intertubular dentin has a lower degree of mineralization than peritubular dentin (Xu and Wang).

The microstructure of dentin changes with aging. Healthy dentin from young teeth has a natural white-yellow color and is opaque due to the light-scattering effect within dentinal tubules (Vasiliadis et al 1983a). Beginning in the third decade of life, an increased mineral deposition within the lumens results in a progressive reduction in tubule diameter. This gradual mineralization of the tubules leads to the appearance of increased translucency with aging dentin (Vasiliadis et al 1983b, Vasiliadis et al 2011). The translucent appearance is due to the mineral substance having a refractive index similar to the rest of the dentin (Thomas et al, Carrigan et al). Dentin will allow light to transmit after an adequate number of lumens have been filled. The resulting transparent-appearing dentin is termed *sclerotic* (Arola et al 2012). Sclerosis initiates in the apical dentin and progresses toward the crown with increasing age (Nalbandian et al). Cross sections of roots show that sclerosis differs in mesiodistal and buccolingual directions, creating a characteristic "butterfly effect" (Russell et al).

The exact mechanism for the formation of intratubular minerals with increasing age in dentin is not fully understood. Results of one study showed that there was minimal evidence of filled tubules in impacted teeth from older patients, which suggests that the physiologic sclerosis is possibly initiated by external stresses imposed by the oral environment (Azaz et al 1977). A contradictory report suggested that the amount of sclerosis increased linearly with age and that it was not markedly affected by the function of the tooth or external stimuli (Weber). Another theory proposed that occlusion of the lumens occurs by dissolution of intertubular crystals and a reprecipitation of new mineral crystallites within the lumens (Porter et al, Kinney et al 2005).

Apoptosis of odontoblasts may be a key factor responsible for the development of physiologic sclerotic dentin (Kabartai et al). A recent study evaluated the role of odontoblast apoptosis in the progression of intratubular mineralization of sclerotic dentin with aging (Yan et al 2022). Healthy teeth were divided into young (≤ 25 years) and old (≥ 60 years). Hematoxylin-eosin (h&e) staining showed that the number of odontoblasts decreased significantly with age, with the most prominent change occurring in the radicular pulp (Fig 14-9). Cleaved caspase-3 staining demonstrated odontoblast apoptosis within the tubules, which was significantly higher in the outer and inner dentin of old teeth. Energy-dispersive x-ray spectroscopy showed that the phosphorous content remained unchanged between the young and old teeth in both the peritubular and intertubular dentin. The peritubular dentin had a greater calcium content (increased from 37.5% to 43.0%) in older teeth, whereas the intertubular dentin had a decreased calcium content (decreased from 48.8% to 39.6%). The stoichiometric ratios of calcium and phosphorous in the young and old peritubular dentin were 1.41 and 1.95, respectively. Scanning-based nanoindentation showed that the old intertubular dentin exhibited a significantly higher elastic modulus. The authors concluded that odontoblast apoptosis, starting at the cell




▲ **FIG 14-9** Representative images of the root canal (middle third) from representative young and old teeth after standard h&e staining. At higher magnification *(right)*, the odontoblast layer in young dentin is evident, whereas old dentin has no obvious odontoblast layer. (Reprinted with permission from Yan et al 2022.)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extension in dentinal tubules and proceeding from outer to inner dentin, contributes to the stoichiometric calcium/phosphorous ratio in peritubular dentin, which is potentially responsible for intratubular mineralization due to an imbalance of calcium and phosphorous ions (Yan et al 2022).

## Age and mechanical properties of dentin

The age-related changes in the microstructure of dentin contribute to the mechanical properties of the tooth. The damage tolerance of the tooth is reduced with age (Yahyazadehfar et al). Coronal dentin becomes more brittle as a patient ages. This causes a significant reduction in both the strength and energy required for fracture. There is a decrease in strength of almost 20 MPa per decade of life that begins shortly after reaching adulthood. There is a 50% reduction in strength and a 75% reduction in energy required to fracture old dentin compared to young dentin (Arola and Reprogel, Arola et al 2012). Furthermore, the fatigue strength (Kinney et al 2005, Ivancik et al 2011), fracture toughness (Koester et al, Nazari et al), and fatigue crack growth resistance (Bajaj et al, Ivancik et al 2012) also undergo a significant reduction with age. The fatigue crack growth resistance of dentin decreases with increasing age, and the incremental rate of crack extension is up to 100 times greater in older patients (Bajaj et al).

Most of the previously mentioned studies focused on coronal dentin (Montoya et al 2015, Montoya et al 2018), but recently more attention has been devoted to radicular dentin (Xu et al, Yan et al 2017). Results from static and cyclic loading revealed a considerable decline in radicular dentin strength with age, with a reduction of roughly 25 MPa per decade on average. There was also a significant reduction in fatigue strength with increased age. Both the mineral-to-collagen ratio and the degree of collagen cross-linking increased with age, with the greatest alterations detected in the apical and middle thirds, respectively (Yan et al 2017). Another study evaluated the effect of root canal treatment on radicular dentin. Dentin from endodontically treated teeth exhibited significantly lower strength than tissue from age- and donor-matched unrestored tooth pairs. Root-filled teeth exhibited a significantly greater collagen cross-linking ratio, above that associated with aging (Yan et al 2019).

Intertubular dentin accounts for more than 90% of the total volume of dentin, and type 1 collagen accounts for about 90% of the intertubular matrix (Nanci). Due to this volume, changes in intertubular dentin and collagen likely have a significant effect on the mechanical properties with increased age. A recent study investigated age-related changes in intertubular dentin and the differences in age-related degradation between vital and pulpless teeth (Yan et al 2020). A total of 12 noncarious single-rooted premolars from 8 patients were selected and divided into young (< 25 years), old (> 60 years), and old nonvital (> 60 years) groups. The four teeth in the old and old nonvital groups were matched pairs of teeth. Scanning mode nanoscopic dynamic mechanical analysis was used on the intertubular and peritubular dentin. There were significant changes in the dynamic moduli of intertubular dentin with age, which were most pronounced in the apical third of the root. The storage modulus of the intertubular dentin, which quantifies the purely elastic resistance to deformation, was significantly larger for both the old vital and nonvital teeth than for the young teeth over the entire root length. The tan delta parameter, which quantifies the relative capacity for viscous deformation, was significantly lower in the two older groups, regardless of location. The authors concluded that radicular dentin has a reduced capacity for viscous deformation with aging and consequently becomes brittle. It appears that removing the pulp accelerates the aging process or exacerbates the degree of degradation (Yan et al 2020).

Collagen cross-linking accompanies the previously discussed microstructural changes in dentin. Because the collagen in bone and dentin have similar composition and properties, reviewing changes in bone collagen with aging is relevant. Collagen undergoes posttranslational modification that involves increased cross-linking (Saito and Marumo). These cross-links can be formed by either enzymatic or non-enzymatic processes (Boskey and Coleman). Glycation is a multistep non-enzymatic reaction between reducing sugars and free amino groups of long-lived proteins such as collagen, resulting in the accumulation of advanced glycation end products (AGEs). Accumulated AGEs bind to collagen and act as a cross-link between collagen fibrils (Haluszka et al). AGEs are also produced by oxidation reactions and form naturally as collagen ages. Therefore, unlike the enzymatically mediated cross-links, AGEs accumulate naturally in bone with age (Burr). Unlike bone, dentinal collagen has no turnover, which may facilitate the progressive accumulation of AGEs in the extracellular matrix over time. As part of the normal aging process, dentinal collagen is subjected to glycation (Haluszka et al). Previous studies have shown the presence of AGEs in aged dentin (Miura et al, Fukushima et al, Shinno et al, Valenzuela et al, Greis et al). There is some evidence that AGE accumulation is predominant in the peritubular area of root dentin (Miura et al, Fukushima et al, Matsuda et al). AGEs have been reported to influence the behavior and mechanical properties of aged dentin (Alania et al). The accumulation of AGEs is responsible for several complications in diabetes mellitus and is implicated in other age-related chronic illnesses (Lopez-Moreno et al, Ruiz et al).

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A recent study (Schuh et al) quantified methylglyoxal (MGO)–derived AGEs in aged dentin. Results showed an increased presence of MGO-derived AGEs in the organic matrix of dentin specimens from patients over 50 years old compared with the specimens from patients under 26 years old in both crown and root. The ultrastructure of collagen in the over-50-year group included diverse areas with a lack of D-banding register and a loss of clear fibril morphology. Atomic force microscopy nanoindentation demonstrated an increase in the elastic modulus of peritubular and intertubular collagen in the over-50-year group (Schuh et al). Therefore, the accumulation of AGEs during aging can affect the structural and mechanical properties of dentinal collagen, resulting in increased collagen cross-linking and stiffer dentin.

The effect of water loss on mechanical properties of dentin has also been recently evaluated. A study used 3D digital image correlation to evaluate the effect of free water loss on dentin. Root dentin displayed an increased formation of heterogenous residual strain with loss of free water, which was accentuated by axial compressive loading, while the resistance to fatigue failure decreased (Nadeau et al). Aged dentin has a lower water content, which may lead to an increase in collagen cross-linking, which in turn causes greater shrinkage, brittleness, and loss of toughness. In this study, partially dehydrated root dentin exhibited the properties of a brittle material with a decreased toughening process. Another recent study showed that water loss and shrinkage strains are significantly greater in the apical third of endodontically treated teeth from patients older than 60 years (Yan et al 2021).

Aged dentin differs from young dentin in structure and mechanical properties. Dentin undergoes a decrease in resistance to fatigue and fracture. These age-related differences may be attributed to intratubular mineralization, changes in peritubular and intertubular composition, increased collagen cross-linking, and the reduced water content. These factors may contribute to the increased incidence of cracks and vertical root fractures in older individuals. Treatment modalities, including assessment of occlusal forces, should reflect these findings. Chapter 12 further investigates the clinical presentation and management of cracks and fractures. CBCT imaging can assist in the preclinical diagnosis of cracked teeth and vertical root fractures through careful observation of the patterns of bone loss (Alaugaily and Azim).

## Age-Related Changes in Cementum

Cementum is a mineralized tissue covering root dentin. Acellular cementum is located on the cervical portions of roots and anchors the primary collagen fibers of the periodontal ligament, providing attachment between the tooth and surrounding alveolar bone. Cellular cementum is located on the apical portion of roots and in furcation regions (Zhao et al).

Cementum is gradually deposited over time, resulting in increased thickness primarily around the root apex. An early study showed that the thickness of cementum approximately tripled between the ages of 11 and 76 years, primarily in the apical region (Zander and Hurzeler). Other studies have confirmed continual cementum deposition with age, particularly in the apical region (Gustafson, Azaz et al 1974, Nitzan et al). These changes in cementum thickness cause an alteration in the apical anatomy of older patients (Kuttler, Stein and Corcoran). Clinicians should be aware of these differences. See chapter 17 for a more comprehensive review of apical anatomy and canal morphology.

As people age, there is an increased prevalence of gingival recession (Heasman et al). Root surfaces can also be exposed by mechanical injury or periodontal surgery. Cementum surfaces have a lower mineral composition and a greater proportion of organic material than enamel surfaces. Exposed cementum surfaces are especially vulnerable to demineralization by plaque acids. A recent investigation of the American population reported that age was associated significantly with root caries and that untreated root caries was most prevalent in those aged 70 years or older (12.5%) and 60 to 69 years old (12.5%) (Bashir). Similar findings were reported in another study (Badr and Sabbah). The management of root caries, including noninvasive options, is a topic of increasing interest as patients retain their teeth longer with aging (AlQranei et al).

## Conclusion

The global population of older adults is rapidly growing due to longer life expectancy and improvements in health care. Significant research demonstrates a direct connection between oral health, whole-body health, and healthy longevity (Fukai et al). Maintaining the oral health of the aging patient population will be the next challenge for dental practitioners and will certainly become a larger aspect of dental practice in the future.

The increased preservation of natural teeth, many of which are already heavily restored, into later adulthood results in complex dental treatment needs. Maintaining natural teeth improves the quality of life, health, and longevity of older individuals (Johnstone and Parashos, Neelakantan et al, Chen et al). Significant technologic and biologic advancements have been made in all disciplines of dentistry, making long-term retention of natural teeth more attainable. Endodontic treatment is an essential part of this process. The procedural objectives of endodontic


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treatment in older patients are the same as those for younger patients. However, calcification may increase the technical difficulty in locating canals (Zilinskaite-Petrauskiene and Haug) (Fig 14-10). CBCT and magnification can serve as useful adjuncts (Kiefner et al, Chaniotis and Ordinola-Zapata).

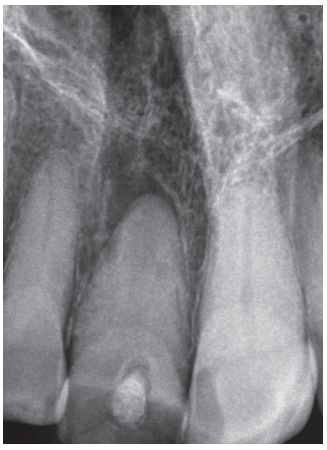
Recently, guided techniques using CBCT and a digital surface scan have been explored for virtual planning of access preparations. Guided endodontics with static or dynamic navigation appears to be a safe and less invasive method for locating calcified root canals (Connert et al). These promising techniques will likely undergo modifications to facilitate usage in the future.

Patient age is not a prognostic factor for nonsurgical root canal treatment. A systematic review of 24 longitudinal studies demonstrated that increased patient age did not decrease the success or survival rates (Shakiba et al). Therefore, dentists and patients should not make treatment decisions based solely on age.

Many factors should be considered in the management of older patients. Their capacity to tolerate dental procedures, cognitive status, and ability to communicate should be evaluated prior to treatment. Additionally, their systemic condition and associated medications may influence treatment-planning decisions, making tooth retention a more favorable option than extraction. When extraction is inevitable, an implant-supported restoration may be an option to consider (Doyle et al).

Future directions may include a more comprehensive understanding of the aging process in general. Clearly there are consequences to dentin due to aging, and more research is anticipated. There is also an increased interest in vital pulp therapy and regeneration for mature teeth (American Association of Endodontists, Nakashima et al).

The objectives of oral health care for this aging patient population should be to treat and prevent oral infection, promote OHRQoL, ensure oral comfort, provide the means to restore oral function when necessary, and maintain an acceptable dental appearance (Müller et al). Understanding the age-related changes to the dentin-pulp complex is important for the clinician to help achieve these goals.



◀ **FIG 14-10** Periapical radiograph of the maxillary anterior area. The maxillary right central and lateral incisors display almost complete calcification of the root canal space.

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# 15

# ODONTOGENIC VS NONODONTOGENIC DIFFERENTIAL DIAGNOSIS OF TOOTHACHE

W. Craig Noblett
Iryna H. Daline
Alan S. Law

According to a recent report from the US Centers for Disease Control and Prevention (Oral Health Tips for Adults), approximately 40% of Americans experienced pain in their mouth in the past year. Toothache is the most common cause of dental pain (Cohen et al), but there are many conditions that can cause pain that mimics a toothache. Thus, it is critical for dental practitioners to understand the potential causes for dental pain and be proficient in the diagnosis of oral pain.

The US physician Martin Fischer (1879–1962) stated, "Diagnosis is not the end, but the beginning of practice." These words certainly relate to the management of orofacial pain. Stated another way, practitioners should not initiate treatment without an informed and appropriate diagnosis. Unfortunately, arriving at a diagnosis for orofacial pain can be particularly challenging, and treatment without a well-formulated diagnosis can result in mismanagement and might exacerbate existing pain.

This chapter provides a background on mechanisms of orofacial pain and explains why diagnosis of orofacial pain can be challenging. In addition, it covers the diagnosis of pulpal and apical pain and conditions that might mimic pulpal and apical pain. The chapter also reviews the recently published *International Classification of Orofacial Pain* (ICOP) (International Classification of Orofacial Pain [ICOP]). The aim of the ICOP is to create a comprehensive, internationally accepted classification that addresses orofacial pain and enhances the research and clinical management of orofacial pain. Practitioners should find it particularly useful when a diagnosis is uncertain. The ICOP is hierarchical, so a practitioner can limit a diagnosis to a more general first or second digit level, which should be sufficient for most practitioners. The general format, showing the first two levels of the ICOP, is shown in Fig 15-1. For pain specialists, the diagnosis may extend to the fourth or even seventh digit. An example of the various levels as they relate to pulpal pain is shown in Box 15-1.

**BOX 15-1 An example of the hierarchical structure of the ICOP codes and diagnoses for pulpal pain**

1. Orofacial pain attributed to disorders of dentoalveolar and anatomically related structures
  1.1 Dental pain
    1.1.1 Pulpal pain
      1.1.1.3 Pulpal pain attributed to pulpitis (pulpal inflammation)
        1.1.1.3.2 Pulpal pain attributed to irreversible pulpitis due to infection of dentin
          1.1.1.3.2.1 Pulpal pain attributed to irreversible pulpitis due to caries extending to the pulp

Modified from Pigg et al 2021.

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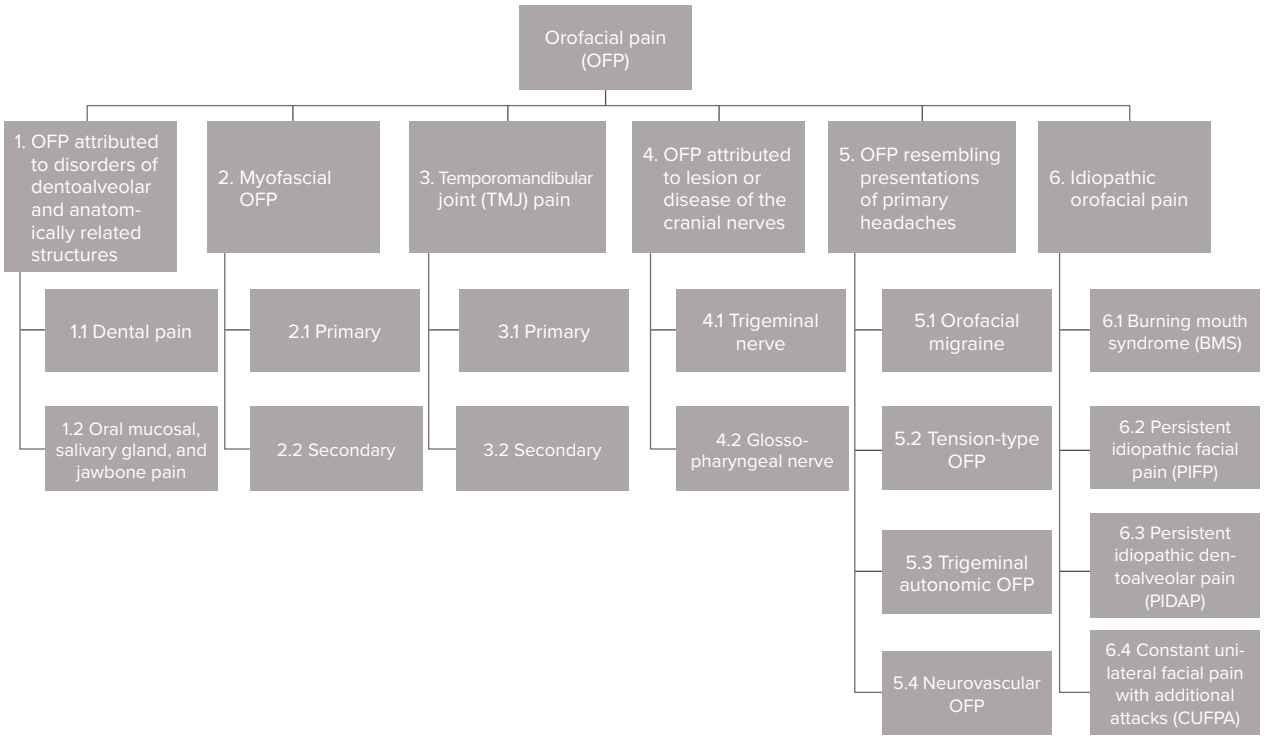
Orofacial pain (OFP)
1. OFP attributed to disorders of dentoalveolar and anatomically related structures
2. Myofascial OFP
3. Temporomandibular joint (TMJ) pain
4. OFP attributed to lesion or disease of the cranial nerves
5. OFP resembling presentations of primary headaches
6. Idiopathic orofacial pain
1.1 Dental pain
1.2 Oral mucosal, salivary gland, and jawbone pain
2.1 Primary
2.2 Secondary
3.1 Primary
3.2 Secondary
4.1 Trigeminal nerve
4.2 Glossopharyngeal nerve
5.1 Orofacial migraine
5.2 Tension-type OFP
5.3 Trigeminal autonomic OFP
5.4 Neurovascular OFP
6.1 Burning mouth syndrome (BMS)
6.2 Persistent idiopathic facial pain (PIFP)
6.3 Persistent idiopathic dentoalveolar pain (PIDAP)
6.4 Constant unilateral facial pain with additional attacks (CUFPA)

▲ **FIG 15-1** The hierarchical structure of the ICOP showing the six main categories of orofacial pain, with subcategories below each main category. There are up to seven levels of detail for each main category, with only levels one and two displayed here. (Modified from Pigg et al 2021.)

## Referral of Pain

The source of pain in the orofacial region can so often be traced to something dental that it is often the first thought that enters the clinician's mind when a patient reports pain in this area. Indeed, the impulse to default to a dental source has nearly risen to the level of being reflexive, leading many practitioners to immediately recommend a referral to an endodontist even prior to a basic examination. It is even more difficult to resist this temptation when presented with a patient who is convinced that they know precisely which tooth is causing their pain. An even more devastating outcome of making this assumption is the scenario where treatment is recommended and completed yet the patient's original symptoms remain unchanged. It is not an uncommon occurrence to meet a patient who has undergone multiple endodontic procedures and even subsequent extractions only to report that their symptoms have remained unchanged or even become worse during the course of treatment. This type of experience is not only humbling for the clinician but can also be harmful and devastating to the patient. The need to avoid this occurrence underscores the importance of a complete examination and accurate diagnosis.

Dental clinicians must keep in mind that not all pain perceived to be felt in the teeth originates in the dental structures. This is the core principle of *referred pain*, where the site at which the patient perceives the pain is different than the actual source of the pain. For this reason, it is wise for the clinician to maintain a healthy skepticism when approaching the diagnostic examination. If referred pain is felt in a tooth, it is designated as a nonodontogenic toothache and can pose a significant diagnostic challenge, so a thorough and complete examination is necessary to correctly identify the source of pain.


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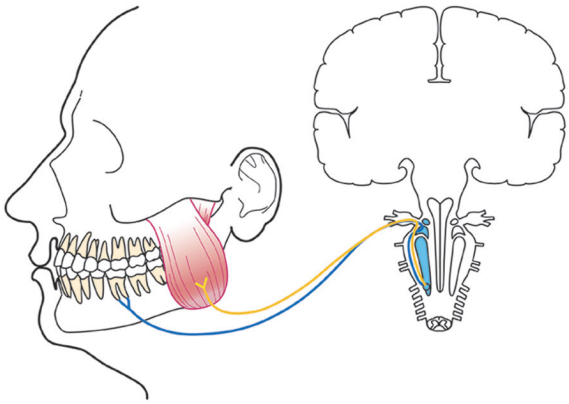
## Theory of convergence

Referred pain is commonly agreed upon as the most common mechanism to explain the nonodontogenic toothache. In the efforts to explain the phenomenon of referred pain, several theories have been proposed, the most widely accepted being the theory of *convergence* (Hargreaves and Keiser) (Fig 15-2). The concept of convergence would seem to be intuitive based on anatomical observations that the number of primary afferent neurons conveying input from peripheral sites far outnumber the second-order neurons in the central nervous system (CNS) (Okeson 2014). This anatomical arrangement would necessitate that primary afferent neurons from multiple peripheral tissues must synapse with a single second-order neuron (Sessle et al). It has been estimated by Sessle that approximately 50% of all pulpal neurons converge with other neurons on the same second-order trigeminal projection neuron (Sessle 1986).

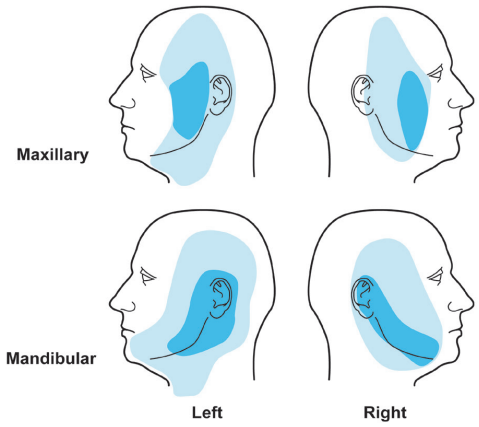
Further complicating this scenario is the phenomenon of central sensitization. This occurs when a constant barrage of nociceptive input causes alteration of the central neurons, resulting in a lower threshold for generating an action potential (Sessle 2000, Kaube et al) and therefore an increased perception of pain.

Understanding the theory of convergence is critical because of the significant clinical implications that help to explain the phenomenon of the site and source of pain not being coincident. This occurs when the patient perceives pain at a specific site, but efforts at reproducing those symptoms are unsuccessful and the perceived site is often devoid of any signs of pain or pathosis. An example is illustrated in Fig 15-2, in which the patient perceives pain in the mandibular molar region and is convinced that it originates from a tooth, but the actual source is a trigger point in the masseter muscle. It is important to keep in mind that any visceral source of pain can result in a referral phenomenon that can occur in nearly any location in the body. In the head and neck region, this can often manifest as pain felt in the teeth. However, it is also important to realize that pain that originates in the pulp can be referred to other craniofacial structures (Wright and Gullickson 1996a, Wright and Gullickson 1996b) and would explain the description often voiced by patients of pain radiating outward from a tooth or even a quadrant. This type of referral is typically confined to the maxillary or mandibular distribution of the trigeminal nerve. For these reasons, it is critical that meticulous diagnostic skills be developed to accurately identify the source of pain.

Another important concept to keep in mind is that as pain intensity increases, the incidence of referral to other sites also increases. Falace et al found that the intensity of pain was the most significant feature associated with referral to other structures, rather than the quality or duration of the pain. In their study, approximately 90% of all patients who reported moderate to severe pain of odontogenic origin also reported pain in adjacent craniofacial structures. Their conclusion was that as the intensity of pain increases, the more likely it is that the patient will report pain in associated structures. Figure 15-3 illustrates the most common referral patterns of pain originating from the first molars that is perceived in adjacent structures.



▲ **FIG 15-2** Convergence of afferent fibers from dental pulp on the same projection neuron in the trigeminal nuclear complex that receives afferent input from the masseter muscle. (Reprinted with permission from Hargreaves and Keiser.)




▲ **FIG 15-3** Extraoral referral patterns of pain originating from maxillary right (n = 27), mandibular right (n = 31), maxillary left (n = 38), and mandibular left (n = 47) first molars. The *dark blue* shading indicates the most frequent areas of pain referral; the *light blue* shading indicates less frequent areas of referral. Referred pain does not generally cross the midline. (Modified with permission from Falace et al.)

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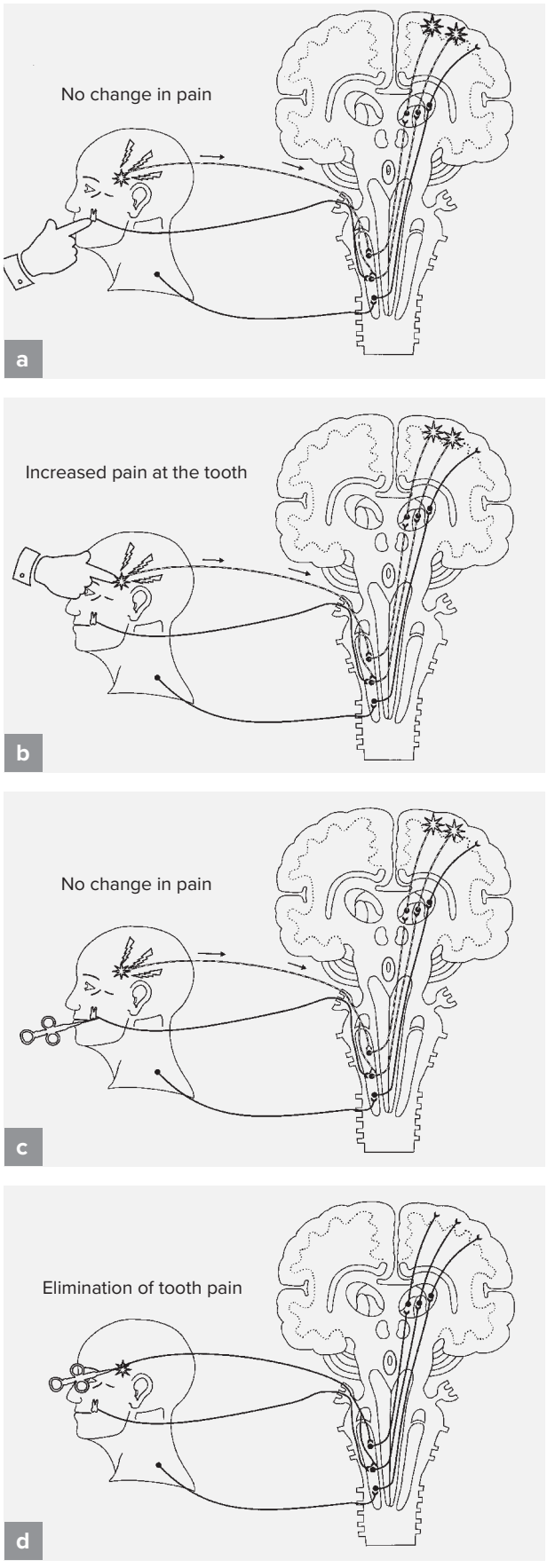
**BOX 15-2 Rules to exclude primary (odontogenic) and confirm referred (nonodontogenic) pain**

1. Local stimulation of the site of pain does not increase the pain.
2. Local stimulation of the source of pain increases the pain at both the source and the site of pain.
3. Local anesthetic blocking of the site of pain does not decrease the pain.
4. Local anesthetic blocking of the source of pain decreases the pain at both the source and the site of pain.

A fundamental responsibility for any clinician is to accurately assess and appropriately treat a patient's chief complaint. In treating those patients presenting with a complaint of pain, every clinician must develop their diagnostic skills and practice a detailed and thorough protocol of data collection and examination so that the source of pain can be properly identified. Central to this process is an appreciation for the wide variety of presentations one will encounter in patients with a complaint of orofacial pain. Understanding that the source of pain may differ from the perceived site of pain as indicated by the patient is critical in order to avoid unnecessary treatment and to accurately address the chief complaint.

## Differentiation between site and source of pain

In determining the etiology of a patient's pain, there are four essential guidelines that can assist the clinician in differentiating between a *site* of pain and a *source* of pain (Box 15-2 and Fig 15-4). The strategic and judicious administration of local anesthetic can be of great assistance in making this determination (Okeson 2014, Okeson and Falace, Falace et al, Schmidt and Wong, Okeson 2008).




▶ **FIG 15-4** Four important clinical rules that help differentiate odontogenic toothache from nonodontogenic toothache. *(a)* Local provocation of the site of pain (a painful tooth) does not increase the pain. *(b)* Local provocation of the source of the pain (a myofascial trigger point in the temporalis muscle) increases the pain not only at the source but also at the site of pain. *(c)* Local anesthesia of the site of pain (the painful tooth) does not decrease the pain. *(d)* Local anesthesia of the source of pain (the temporalis trigger point) decreases the pain not only at the source but also at the site of pain (tooth pain is eliminated). (Reprinted with permission from Okeson 2014.)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### Local stimulation of the site of pain does not increase the pain

If a tooth is suspected to be the source of pain, then applying the appropriate stimulus to the suspected tooth should either trigger the pain or increase the pain level. Thermal allodynia is considered the defining feature for detecting pain of pulpal origin, while mechanical allodynia is considered to be the definitive feature of periapical/periradicular pain. If the suspected tooth is indeed the source of pain, applying these stimuli should replicate or intensify the pain that constitutes the patient's chief complaint (Klausen et al). When these stimuli do not reproduce the pain as reported by the patient, the clinician must consider a nonodontogenic source. In this scenario, because the perceived source of pain (a tooth) is not the actual location of nociceptor activation, application of stimuli to the suspected tooth will not elicit or change the pain, supporting the possibility of a nonodontogenic toothache.

### Local stimulation of the source of pain increases pain at both the source and the site of pain

When a "toothache" is thought to be a site of referred pain, the source can sometimes be located with relative ease, such as the example of a myofascial trigger point. Once identified, a trigger point should be stimulated and the patient asked if the pain is felt in any other location. If the trigger point is indeed the source, then the patient will often report pain at the site of palpation and radiating pain to the originally suspected site, the tooth. This finding should confirm a clinician's suspicion that the reported tooth pain is referred from a different source and should assist in targeting treatment or therapies to the appropriate site, thereby avoiding unnecessary endodontic treatment.

Some sources of pain that result in nonodontogenic tooth pain are not easily located or reproduced, making the connection difficult to demonstrate clinically. In this case, gathering a thorough history should assist in establishing a diagnosis. Strategic questioning of the patient regarding those factors that exacerbate the reported tooth pain can be valuable in identifying the actual source of pain. In addition, understanding the characteristics of the pain such as quality, duration, and onset may also provide the clinician with important clues as to the true pain source.

### Local anesthetic blocking of the site of pain does not decrease the pain

Administration of a local anesthetic can be an extremely valuable method in identifying the source of reported tooth pain. When a tooth is indeed the primary source of pain, providing anesthesia appropriate to the location of the tooth should greatly reduce or eliminate the pain. In this event, the clinician will need to identify the local cause of nociception, whether pulpal, periradicular, periodontal, or mucosal, and provide the appropriate treatment for the condition.

If administration of anesthetic is ineffective at eliminating or reducing the pain, referred pain must be considered. One pitfall in making this assumption is inadequate assessment of the efficacy of the anesthetic. Simply asking the patient if they feel numb may result in a false negative response where the tissues in the field of anesthetic administration are indeed anesthetized, but the patient still feels the pain and thus they perceive this as a failure of the anesthetic. A more effective question would be to ask: "I know you might feel numb, but does it still hurt?" If the patient answers, "I feel very numb, but there is no change in the pain," then the clinician has reason to believe that the tissues in the field of anesthesia are not the source of nociception and therefore should not be the target of any treatment. The use of local anesthetic in this way is an important method to differentiate between dental pain and referred pain from another source. This should be employed in any situation when the source of pain is in question.

Those patients presenting with severe pain in mandibular molars may not experience complete anesthesia following an inferior alveolar block. Nusstein et al found a success rate of only 38% among patients presenting with irreversible pulpitis associated with mandibular molars. This scenario might mislead a clinician to mistakenly identify the poor response to anesthesia as an indication that the pain is referred from a different source. In those situations where sensibility testing is equivocal and anesthesia is indicated to aid in diagnosis, incomplete response to anesthesia may occur, so a supplemental injection such as a buccal infiltration or intraosseous injection should be given, as this can increase the success of anesthesia to 88% (Nusstein et al). The combined injections in this type of case should provide a relatively high level of confidence with respect to differentiating odontogenic and nonodontogenic sources of pain.

### Local anesthetic blocking of the source of pain decreases the pain at both the source and the site of pain

When an odontogenic source has been ruled out by the anesthetic test, the true source needs to be identified. When a nonodontogenic source is identified, for example a myofascial trigger point, the source should be anesthetized, if possible, for confirmation. In the case of a myofascial trigger point, that would involve a trigger point injection. Anesthesia of the true source will eliminate not only the pain at the true source but also any pain at the site of referred pain. When this occurs, the clinician has identified the true source of pain and any treatment can then be directed at that true source.

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**BOX 15-3 Common features of odontogenic dental pain**

- Ability to reproduce the patient's chief complaint
- Relief of pain after administration of local anesthetic
- Unilateral pain
- Etiologic factors present (caries, defective restorations, cracks)
- Pain to temperature change (pulpal origin)
- Pain to percussion (periradicular origin)
- Localized pain (periradicular origin)

Although employing local anesthetic can be very informative, false positive results are possible and must be considered as a possible outcome. Therefore, the local anesthetic test should be considered along with all other findings in each case, much like any other diagnostic examination. Typically, the local anesthetic test will provide only an approximate location, because multiple teeth are often anesthetized by most injection techniques (Replogle et al, Kim). In all diagnostic endeavors, it is important to reproduce the patient's chief complaint, but if the complaint cannot be reproduced, alternative origins of pain must be considered, even those teeth already endodontically treated (Keir et al).

## Odontogenic Toothache

Thankfully for clinicians, a large majority of patients presenting with the complaint of dental pain have symptoms that originate from a dental source. The typical diagnoses encountered with odontogenic pain are reversible pulpitis, irreversible pulpitis, acute apical periodontitis, or acute apical abscess. These normally are due to deep caries or defective restorations but can also be caused by cracks, trauma, or recent restorative treatment. When the diagnosis is not straightforward or there are no evident clinical signs of etiology, the clinician must formulate a differential diagnosis to help guide the examination and testing to arrive at a definitive diagnosis.

It should be noted that patients with pain that is strictly of pulpal origin may have a great deal of difficulty in localizing the source to a specific tooth. Once the inflammation spreads to the apical tissues, localization becomes much more predictable. McCarthy et al found that patients with a diagnosis of acute apical periodontitis could correctly identify the affected tooth 89% of the time, while those patients who exhibited strictly pulpal symptoms could only identify the correct tooth 30% of the time. The most common features of pain originating from an odontogenic source are listed in Box 15-3.

## NONODONTOGENIC TOOTHACHE

Any student reading this textbook is likely well acquainted with consuming large amounts of caffeine to keep going and to help meet stressful deadlines. Unfortunately, both stress and a high caffeine intake can increase muscle tension in the face and cause teeth clenching. Such activity puts stress on the masticatory muscles and teeth and leads to jaw pain that can refer to the teeth. This is a tangible and relatable example of how a nonodontogenic toothache can start.

A majority of patients experiencing tooth pain have a clear odontogenic etiology for their symptoms (Currie et al, Daline et al 2023). However, it is common for other types of orofacial pain either to completely mimic tooth pain or to be a part of the "toothache" chief complaint as a comorbid pain condition. In fact, a significant proportion (11% to 28%) of endodontic patients with a history of tooth pain have nonodontogenic pain either as the only etiology for their pain or as a component of their pain (Daline et al 2023, Linn et al).

The ability to diagnose nonodontogenic pain is important not only for initial diagnosis for the "tooth pain" complaint but also to prevent persistent pain after root canal treatment. Research shows that 5% to 10% of patients experience some form of pain 6 months or later following root canal treatment (Jonsson Sjogren et al, Nixdorf et al 2016, Nixdorf et al 2010, Vena et al), and about a fifth of these patients continue to have persistent pain for many years (Daline et al 2020). Over half (53% to 62%) of patients presenting for endodontic reevaluation after root canal treatment with a complaint of persistent tooth pain have a non-endodontic cause for their pain (Nixdorf et al 2015, Vena et al). Another study followed up on patients who had reported persistent pain 6 months after root canal treatment and showed that such patients often have a mixture of odontogenic and nonodontogenic pain etiologies, with temporomandibular disorders and/or headaches being comorbid conditions or causes of their persistent "tooth" pain (Daline et al 2020).

Therefore, recognizing underlying nonodontogenic or mixed (odontogenic and nonodontogenic) pain etiology is of paramount importance to reduce the chances of incorrect or incomplete diagnosis for the pain. This will help avoid postoperative patient concerns, unnecessary additional consultations, and unnecessary dental procedures; minimize the chances of the patient developing persistent pain; prevent delays in appropriate care; and improve the patient's quality of life.

An exhaustive pain history is key to the diagnosis. History-taking should involve a structured clinical interview with guided questions to obtain detailed information about the patient's pain as well as its potential contributing factors.


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More often than not, a thorough and in-depth history alone can provide information sufficient for provisional diagnosis, and clinical examination can be used simply to confirm or refute it. Below are the 10 important domains of the comprehensive pain history, with examples of questions that clinicians can ask their patients in their history-taking process:

1. **Onset, precipitating event, and progression:** How long ago did your pain start? Do you recall what might have triggered it at that time? Has it gotten better, worse, or remained the same since it started?
2. **Location:** Can you point with your finger to the area where you feel the pain? Where else do you feel it? Does the pain radiate anywhere?
3. **Frequency:** Is your pain constant or does it come and go? If it comes and goes, how frequently do you experience this pain?
4. **Duration:** How long on average do the episodes of pain last (seconds, minutes, hours, days, pain never stops)?
5. **Quality:** How would you describe your pain? For example, is it dull, achy, sore, throbbing, pulsating, sharp, shooting, stabbing, electric shock–like, burning, pressing, or some other description?
6. **Intensity (now, average, and worst on 0–10 scale):** How would you rate the intensity of your pain on a scale from 0 to 10, where 0 represents "no pain" and 10 represents "worst pain imaginable"? How would you rate your pain right now, on average, and when it is at its worst?
7. **Aggravating/triggering factors:** What makes this pain worse or triggers it? Would any of the following exacerbate it: hot or cold foods/liquids, biting, chewing, touching or pressing on the tooth or the gums, opening the mouth, clenching or grinding teeth, stress, working, exercising, changing posture (lying down, bending over), lack of or irregular sleep, skipped meals, certain foods, weather changes, menstrual cycle, lightly touching the face, shaving, wind, swallowing, or something else?
8. **Alleviating factors:** What makes this pain better? For example, drinking cold water, brushing teeth or using mouth rinses, prescription pain medication(s), over-the-counter pain medication(s), prescription antibiotic(s), prescription steroid(s), prescription muscle relaxant(s), topical anesthetic applications, caffeine, heat and/or ice compresses, resting/relaxing, mouthguard, jaw massage, being in a quiet and dark room, sleeping, something else, or nothing?
9. **Associated symptoms:** Do you have any symptoms associated with your pain? For example, swelling, drainage from the gums, feeling of the tooth being "too high," fever and/or malaise, pain wakes you at night, pain in other teeth, jaw pain, headache, neck pain, feeling of the bite being "off," jaw clicking/popping/grating noises, jaw locking, jaw fatigue, light/noise sensitivity, nausea/vomiting, nasal congestion or runny nose, tearing, redness or swelling of the eye on the side of pain, vesicles or lesions on the face or inside your mouth, sinus drainage, or something else?
10. **Previous treatments and their effects:** What treatments have you had to alleviate this pain? How has your pain changed due to these treatments? For examples, has it gotten better, has it improved but then come back, has it not changed, or has the pain worsened after the treatment?

In addition to the pain history, it is important to inquire about and recognize potential contributing factors to the pain presentations. These can typically be found by investigating the patient's medical history, medications, sleep quality, and psychosocial factors. The patient's medical history must be assessed for the presence of conditions with a potential to affect orofacial pain presentation, such as sinusitis, migraine or other headaches, fibromyalgia, chronic neuropathic pain, cardiovascular disease, stroke, cancer, herpes zoster infection, rheumatoid arthritis, other autoimmune disorders, and obstructive sleep apnea. A complete list of the medications that the patient takes for their pain (the type of medications and the frequency of their use) as well as any stimulants or selective serotonin reuptake inhibitors may also reveal important clues toward the nonodontogenic pain diagnosis. Further, psychosocial history is often contributory and plays an important role in the development and maintenance of chronic pain as well as the treatment prognosis (Ohrbach and Dworkin). The patient should be asked about their occupation; marital status; caffeine intake; any diet restrictions; smoking, alcohol, and substance use; stress levels; any symptoms or diagnoses of anxiety, depression, posttraumatic stress disorder (PTSD), bipolar disorder, or suicidal ideation; and sleep quality or lack of sleep.

Finally, patients need to be asked about their oral parafunctional habits. Oral habits are important contributing and perpetuating factors for myofascial and temporomandibular joint (TMJ) pain (which may present in or refer to the teeth) and include teeth clenching and/or grinding, gum chewing, lip/cheek/object biting, resting jaw in hand, unilateral chewing, jaw posturing, bite checking, pressing with the tongue on the tooth, and stomach sleeping.

These collected signs, symptoms, and contributing factors can then be associated with one of the four major clusters of nonodontogenic diagnostic entities: musculoskeletal pain, neurovascular or headache pain, neuropathic pain, and nociplastic pain. Descriptive data on these four

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clusters of nonodontogenic pain can be found in Table 15-1 and will help the clinician to generate the initial diagnostic hypothesis.

Further, the recently developed *International Classification of Orofacial Pain* can be used as a helpful reference for diagnostic criteria of specific painful conditions within each cluster of orofacial pain conditions (ICOP). A recent article in the *Journal of Endodontics* provides an overview of the specific ICOP diagnoses that are most pertinent to endodontic practice (Pigg et al 2021) (see Fig 15-1).

**TABLE 15-1** Distinguishing characteristics of four major clusters of nonodontogenic pain disorders that can be gathered using a structured clinical interview

| Structured pain history / Cluster of pain disorders | Onset and precipitating event | Location | Quality | Intensity 0–10 (avg/max) | Frequency | Duration |
|---|---|---|---|---|---|---|
| **Musculoskeletal** (eg, TMD—myofascial pain and/or TMJ arthralgia; muscle spasm; cervical myofascial pain) | Stressful life event(s), endodontic pain, prolonged/traumatic dental procedure | Jaw, temple(s), ear(s), teeth (usually posterior), neck, forehead | Dull, aching, pressure, soreness, tightness, occasionally sharp | 3–10/10 | Usually later in the day (oral habits), occasionally worse upon awakening (sleep bruxism) | Hours to continuous |
| **Neurovascular** (eg, chronic migraine; medication-overuse headache; trigeminal autonomic orofacial pain) | History of headache that changed, stressful life events, head injury, pain medication overuse | Half of the head, forehead, temples, periorbital, sinuses, teeth (usually maxillary) | Throbbing, pulsating, sharp, stabbing, pounding | 6–10/10 | Several times a month, multiple episodes/day, annual/biannual, or continuous | Seconds to hours, hours to days, weeks to months, continuous |


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| Triggers | Aggravating factors | Alleviating factors | Associated symptoms | Previous treatments and their effects | Contributing factors |
|---|---|---|---|---|---|
| Biting, chewing; opening mouth; yawning; talking, clenching, or grinding teeth; dental procedures | Biting, chewing; opening mouth; yawning; talking, clenching, or grinding teeth; stress; poor posture; dental pain, dental procedures | OTC pain meds, heat/ice compresses, resting/relaxing, sleeping, mouthguard, jaw massage | Dental cracks or jaw, tooth, ear, head, neck pain; "swelling" (muscle spasm); limited mouth opening; recent bite change; jaw joint clicking/popping/grating noises or locking | Occlusal adjustments or dental treatment (fillings, RCTs, crowns) with temporary or no relief or even worsening of the pain | Teeth clenching, grinding, gum chewing, lip/cheek biting, resting jaw in hand, hard foods, unilateral chewing, stomach sleeping; rheumatoid arthritis, Ehlers-Danlos syndrome, fibromyalgia; stress, anxiety, PTSD; uneven/unbalanced mouthguard, oral appliance for sleep apnea |
| Barometric pressure changes; menstrual cycle; stress; inconsistent daily schedule (meals, sleep); dehydration; diet; alcohol; light touch; nitroglycerin | Lights, noises, physical activity, bending over; depression, anxiety, stress | Resting/relaxing, being in a quiet dark room, sleeping, pain medications | Photo-/phonophobia, nausea/vomiting, fatigue, yawning, cravings, mood change, neck pain, aura; unilateral conjunctival injection; tearing; eyelid edema; miosis; ptosis; nasal congestion; rhinorrhea; facial sweating; fullness in the ear; restlessness/agitation | Treatments for trigeminal neuralgia or jaw, dental, or sinus pain with unfavorable outcomes; often overuse of pain medication | Lack of sleep/oversleeping; excessive caffeine; skipped meals/overeating; diet (cured meats, aged cheese, citrus, chocolate, caffeine, wine, MSG, nitrates, aspartame); overuse of OTC analgesics (especially Excedrin [contains caffeine]), opioids, benzodiazepines, triptans, barbiturates (> 2d/wk > 3 mo); other chronic pain conditions (TMD, fibromyalgia); stress, anxiety, depression, PTSD; alcohol, substance abuse; family history of migraine or other headaches |

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**TABLE 15-1** *(cont)* Distinguishing characteristics of four major clusters of nonodontogenic pain disorders that can be gathered using a structured clinical interview

| Cluster of pain disorders / Structured pain history | | Onset and precipitating event | Location | Quality | Intensity 0–10 (avg/max) | Frequency | Duration |
|---|---|---|---|---|---|---|---|
| Neuropathic | **Episodic** (eg, trigeminal neuralgia [TN], glossopharyngeal neuralgia [GN]) | Sudden, no precipitating event | Does not cross the midline. TN: along mainly second or third division of CN V GN: ear, base of the tongue, tonsillar fossa, under angle of the jaw | Shooting, electric shock–like, cutting, burning | "11"/10 ("suicidal") | Only during waking hours, provoked by triggers or sometimes spontaneous | Seconds to 2 minutes, remission periods last days to years |
| | **Continuous** (eg, posttraumatic trigeminal neuropathic pain | Within 6 months after trauma or invasive treatment (RCT, implant, anesthetic injections, extraction) resulting in an identifiable peripheral trigeminal nerve lesion | Unilateral facial or oral pain that occurs after accidental or surgical injury within the dermatome of the affected nerve; does not cross the midline | Dull, deep, burning, shooting, squeezing, prickling, itching, numb | 5–8/10 | Daily, continuous; intensity may vary throughout the day | Continuous, lasting > 3 months |
| **Nociplastic** (eg, persistent idiopathic dentoalveolar pain) | | No causative event* (*There may be a history of previous trauma to the affected area; however, no signs of local cause can be identified.) | Intraoral dentoalveolar region (tooth/teeth, gingiva[e], bone); occasional difficulty localizing pain, which can affect multiple teeth | Deep, dull, pressure-like, burning or electric shock–like; numbness or tingling sensations | 5–10/10 | Daily, recurring, or continuous; may have exacerbations, but does not disrupt sleep | > 2 hours/day for more than 3 months; unchanging over the months |

## Nonodontogenic Toothache of Musculoskeletal Origin

The cluster of musculoskeletal orofacial pain consists of temporomandibular disorders (TMDs) and cervicalgia. TMDs are a group of musculoskeletal and neuromuscular conditions involving masticatory muscles, TMJs, and all associated tissues (De Leeuw et al). Painful TMDs include TMJ arthralgia (joint pain), myalgia (muscle pain), and headache attributed to TMD (Schiffman et al). Myalgia is further divided into local myalgia, myofascial pain, and myofascial pain with referral (Schiffman et al) (Fig 15-5). Myalgia and arthralgia are the most relevant conditions to be considered in the differential diagnosis of tooth pain. The masticatory muscles, TMJs, temporalis tendon, and cervical muscles have all been reported to refer pain to the teeth (Daline et al 2023, Duffin et al, Farella et al, Fernández-de-Las-Peñas et al, Fricton et al, Kalladka et al, Wright).

Myofascial trigger points have been implicated in pain referral from the muscles and are defined as "hyperirritable spots in skeletal muscle that are associated with a


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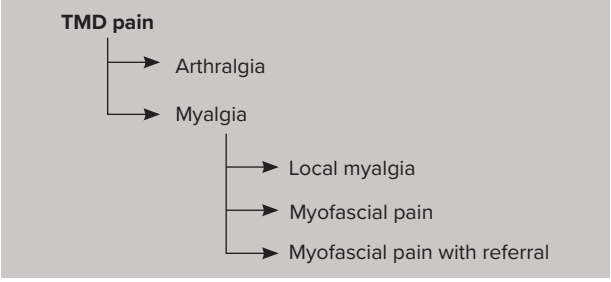
| Triggers | Aggravating factors | Alleviating factors | Associated symptoms | Previous treatments and their effects | Contributing factors |
|---|---|---|---|---|---|
| TN: washing or touching face, shaving, talking, brushing teeth<br>GN: swallowing, chewing, talking, yawning | TMD, dental/ endo pain, other pain, stress, anxiety, dental treatments | None; sometimes hard pressure over the trigger zone | Sometimes preceded by a period of dull continuous toothache (pretrigeminal neuralgia); continuous background pain between the attacks; cluster headache – TN, MS–T | Dental treatments with unfavorable outcomes | Other pains in the orofacial region (dental, TMD); secondary TN: intracranial mass, lesion, aneurysm, or vascular malformation |
| N/A, constant | Stress, fatigue, illness, repeated dental procedures (reinjury to the nerve) | Rest in mornings; topical anesthetics, analgesics, certain anticonvulsants or antidepressants (if trialed) | Feeling of swelling, foreign body sensation, hot or cold feeling in the area; history of and clinical abnormal somatosensory findings: numbness, hyperalgesia, paresthesia, allodynia, dysesthesia; tissues appear healed from the previous injury | Lack of response to anti-inflammatory medications; pharmacologic and dental treatments with little to no relief | Invasive dental treatments causing repetitive nerve injury |
| N/A, constant | Stress, repeated dental procedures | Relaxing, systemic and topical pharmacotherapy for neuropathic pain (if trialed) | No local pathologies, normal clinical or radiographic findings; fatigue, sleep disturbances, memory problems, mood changes | Multiple irreversible dental treatments, anti-inflammatory drugs, opioids, surgery, or injections with little to no pain relief; local anesthesia fails to alleviate the pain | TMD, migraine, tension-type headache, other chronic overlapping pain conditions (eg, fibromyalgia, chronic low back pain, IBS), psychiatric disorders |




▲ **FIG 15-5** TMD pain diagnoses of significance/interest to endodontists.

hypersensitive palpable nodule in a taut band" (Simons et al). There is no "gold standard" diagnosis for trigger points; however, a systematic review by Li at al revealed the most common criteria studies use to diagnose the presence of myofascial trigger points as the following: spot tenderness, referred pain, local twitch response (within the muscle itself upon pricking [Simons et al]), pain recognition (familiar pain), limited range of motion, and/or jump sign (the patient physically jumps or responds to palpation of the area [Fricton et al]). Several of these criteria are often used in combination (Li et al).

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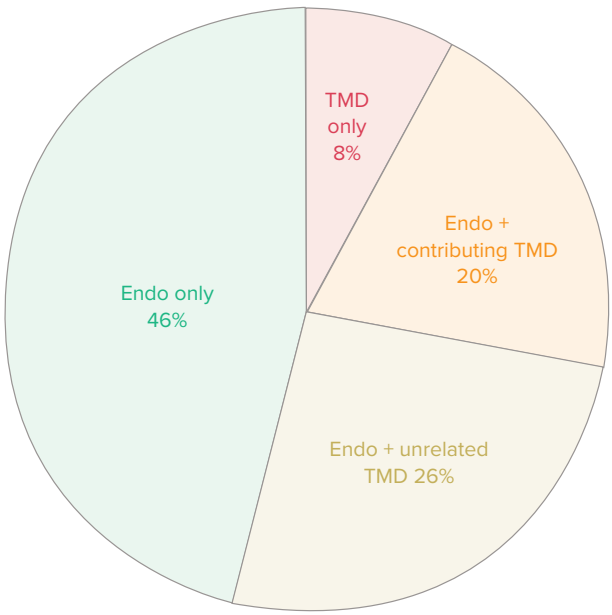
## Myofascial orofacial pain and TMJ pain

Masticatory myalgia and TMJ arthralgia are common in the general population, with a prevalence of about 11% (Janal et al, Manfredini et al 2011). TMD is the most common non-endodontic pain for which patients are mistakenly referred for root canal treatment (Linn et al). Furthermore, TMD pain was found in 54% of patients with a history of tooth pain seeking root canal treatment for their pain (Daline et al 2023). In most of these patients, TMD was present together with endodontic pathosis as a second, comorbid pain condition. However, in 20% of these patients, TMD contributed to and was a component of their chief pain complaint. Even though endodontic treatment may successfully resolve the pulpal-periapical pathosis, these patients could continue to have lingering TMD pain after the treatment, which they might interpret as persistent "tooth" pain. This may lead to postoperative patient concerns, additional appointments, radiographs, CBCTs, unnecessary and ineffective occlusal adjustments, root canal retreatments, apical surgeries, or extractions. Importantly, 8% of patients presenting for endodontic evaluation and treatment with a history of tooth pain had TMD as a sole cause of pain (Daline et al 2023) (Fig 15-6). Performing endodontic treatment in such patients will not address their pain and will lead to persistent "tooth" pain of TMD origin (Daline et al 2023). It is not surprising that another study found that TMD was by far the most common non-endodontic reason for persistent pain after root canal treatment (Nixdorf et al 2015).




▲ **FIG 15-6** Combinations of painful TMD and endodontic pain among patients seeking nonsurgical root canal treatment for their pain (n = 100). (Modified from Daline et al 2023.)

Higher TMD prevalence among endodontic patients was found to be associated with moderate or severe current pain intensity, daily or near daily pain, first pain symptoms that started more than 8 months ago (addressed or unaddressed in that time frame), pain in more than just one tooth, pain to tooth palpation and percussion, use of pain medications, moderate to severe anxiety and/or depression, and high stress (Daline et al 2023). This poses a diagnostic challenge: Patients who have TMD pain typically present with more severe symptoms and signs of tooth pain. Another challenge is that patients do not distinguish well between tooth pain and jaw pain and often aren't aware of jaw pain unless they are specifically asked about its symptoms or until the TMD examination is performed. These factors will naturally steer a clinician's attention away from the jaw muscles and joints to the teeth and may pressure both patients and clinicians toward root canal treatment, leaving TMD-related pain unaddressed. The solution is to screen all patients with recent history of toothache for TMD.

It is important to stress that pain to tooth percussion is not a pathognomonic sign of periapical pathosis of endodontic origin. Tenderness to percussion (on the isolated tooth or multiple teeth) is very common among patients with TMD without any clear odontogenic etiology or clinical or radiographic signs of pulpal-periapical disease (Daline et al 2023). Such tenderness to percussion may be attributed to allodynia, similar to that seen in cases of maxillary sinusitis or migraine headache, as well as central sensitization, pain referral, and potential microtrauma to the periodontal ligament from clenching and grinding on the tooth.

Patients experiencing TMD pain are likely to present with the following pain characteristics (Daline et al 2023, Fricton et al, Wright):

- Patients report a history of symptoms that have been present for several months, described as dull achy pain and occasionally sharp and throbbing pain in one or several, usually posterior, teeth.
- The pain could be bilateral or migrating, meaning that the pain may present on different sides at different times.
- The pain can range in intensity from mild to very severe and last for several hours. It is aggravated by biting, hard foods/gum, opening the mouth wide, clenching or grinding teeth, other oral habits, and stress.
- Pain medications, jaw massage, resting the jaw, and relaxing typically relieve the pain.
- When specifically asked, patients will report additional jaw pain and sometimes headaches, earache, neck pain, "swelling" in the cheek/jaw (pointing to the masseter bulk), or their bite feeling off.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- Over the course of months of pain, patients typically undergo treatments directed to the teeth, such as occlusal adjustments, root canal treatments and retreatments, crowns, and extractions, which provide partial or temporary relief.

Identifying some of these characteristics in the pain history should prompt a clinician to evaluate the patient for TMD.

Evaluation and diagnosis of myalgia and/or arthralgia should be based on the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) (Schiffman et al). The DC/TMD provides validated diagnostic criteria for the diagnosis of arthralgia, myalgia, and myofascial pain with referral, with sensitivities and specificities of 89%/98%, 90%/99%, and 86%/98%, respectively (Schiffman et al). To summarize, the following three conditions should be met to establish the myalgia/arthralgia diagnosis:

1. History of jaw/face pain in the last 30 days AND
2. Pain is aggravated (or alleviated) by jaw function or parafunction AND
3. Familiar pain is reproduced by maximum mandibular opening (or lateral/protrusive movements for diagnosis of arthralgia) OR TMJ/masseter/temporalis muscle palpation

Maximum mouth opening is considered within normal limits if it measures about 40 mm, produces no pain, and does not cause deviation or deflection of the mandible. If it is painful, areas of pain should be noted. In accordance with the DC/TMD examination protocol (Ohrbach et al), palpation should be performed by applying 5 seconds of sustained pressure with one finger over each part of the muscle/TMJ. The temporalis muscle (posterior, middle, and anterior), masseter muscle (origin, body, and insertion), and the area around the lateral pole of the TMJs are palpated using 2 lb of pressure, whereas the lateral pole of the TMJs and smaller supplemental masticatory muscles (posterior and anterior digastric, medial and lateral pterygoid, and temporalis tendon) are palpated using 1 lb of pressure.

Palpation should be performed with the patient's jaw in a relaxed position (Fig 15-7). However, to help identify the borders of the temporalis and masseter muscles (Figs 15-7a and 15-7b), the patient can be asked to clench their teeth and relax a few times as the clinician places their fingers over the temples and cheeks. The TMJ (Fig 15-7c) is located right in front of the tragus of the ear. To assist in locating the TMJs, the patient should open wide and close their mouth to allow the clinician to feel for the translation of the condyle. The posterior digastric muscle (Fig 15-7d) is located below the angle of the mandible. The medial pterygoid muscle (Fig 15-7e) can be palpated extraorally by applying pressure behind the ramus of the mandible or intraorally by sliding a pad of the index finger over the ramus of the mandible behind the last molar tooth, posterior to where an inferior alveolar nerve block would normally be administered. To palpate the lateral pterygoid area (Fig 15-7f), the patient is asked to open only slightly and slide their jaw to the side of palpation, and the examiner's little finger is placed intraorally lateral to the maxillary posterior teeth and advanced posteriorly, superiorly, and medially to apply pressure on the soft tissue overlying the muscle at the typical site for administration of a posterior superior alveolar nerve block. Palpation of the lateral pterygoid area is sensitive and frequently gives a false positive response, so it is important to verify with the patient if the pain they feel is familiar. Temporalis tendon (Fig 15-7g) palpation is performed with the patient's mouth in a maximum opening position; the clinician's forefinger locates the anterior border of the ramus and slides along it as far superiorly as possible to palpate the attachment of the tendon to the coronoid process. The anterior digastric muscle (Fig 15-7h) is palpated below the anterior inferior surface of the mandible. A forefinger of another hand may be placed intraorally on the opposite side of the muscle, and to help identify it, the patient is asked to swallow to note the contraction of the muscle. Cervical muscle palpation is not part of the TMD examination; however, cervical myofascial pain is common along with masticatory myofascial pain, and referral is possible to the jaw and teeth. The sternocleidomastoid muscle (Fig 15-7i) is palpated using a pinch grip in several areas along the muscle from its insertion at the mastoid process to its origin on the clavicle and sternum. The upper trapezius muscle (Fig 15-7j) is palpated from the acromion to the border of the neck. More palpation pressure (4 lb) should be applied to the cervical muscles.

If palpation produces pain, the clinician must verify whether it duplicates/replicates the patient's "familiar" pain, meaning the pain is similar to what the patient has experienced in the past 30 days, regardless of its intensity. If the answer is yes and this pain is modified by jaw function or parafunction, TMD diagnosis is established. In such cases, any pain referral patterns should be noted as well. The patient should be asked if this pain refers or is felt anywhere besides the place of the finger pressure, and if so, where. If the pain refers to the painful tooth/teeth or replicates at least a component of their "tooth" pain complaint, TMJ arthralgia and/or myofascial pain with referral is then confirmed as at least a contributing factor to the patient's pain complaint. These referred pains tend to follow some generalized patterns (Daline et al 2023, Simons et al, Wright) (see Fig 15-7), although clinical judgment is always required. If a clinician identifies

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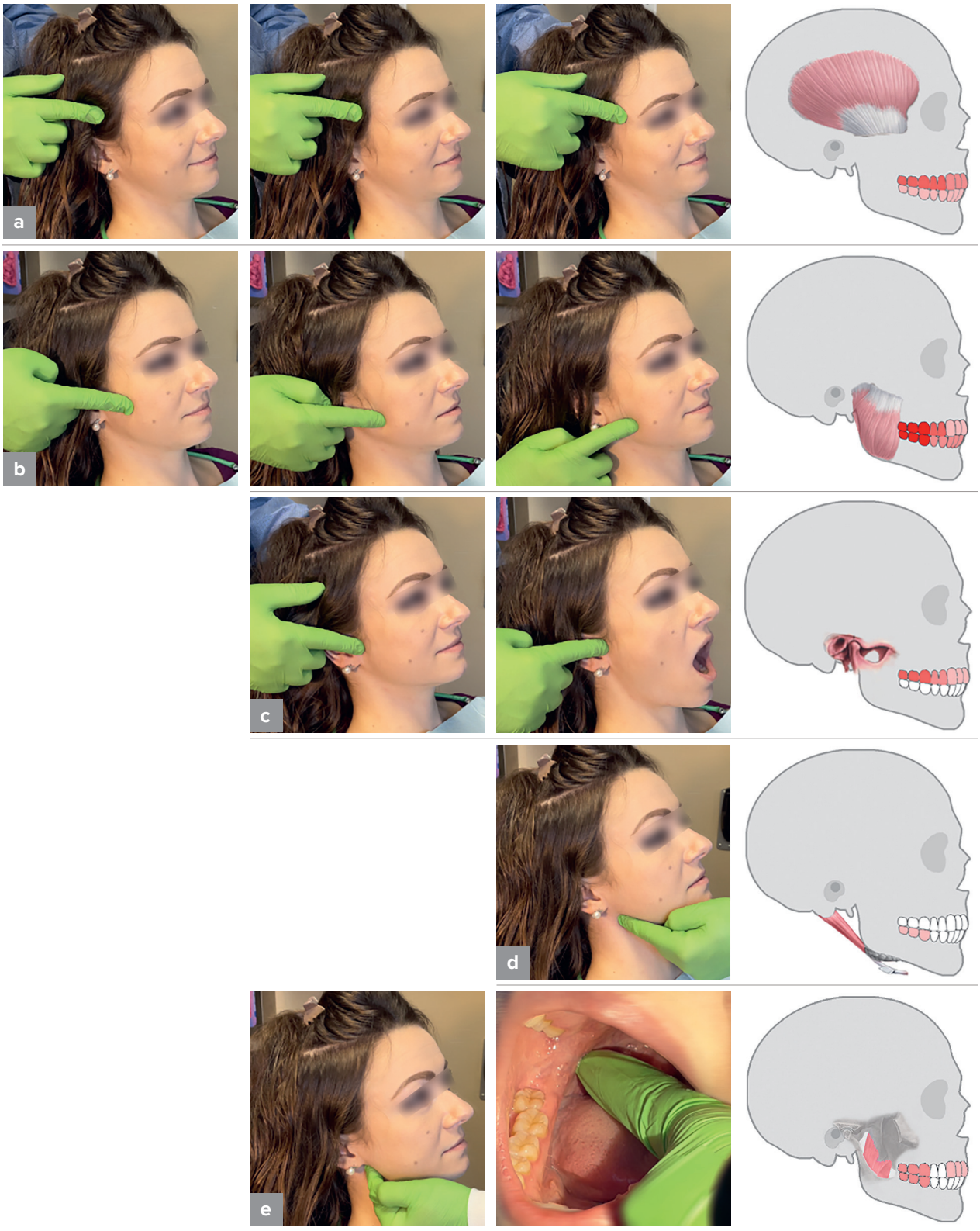


▲ **FIG 15-7** Demonstration of the palpation sites and the typical referral patterns of musculoskeletal pain to teeth (the darker the red color of the teeth, the more common the pain referral to those teeth). *(a)* Temporalis muscle. *(b)* Masseter muscle. *(c)* TMJ. *(d)* Posterior digastric muscle. *(e)* Medial pterygoid muscle.


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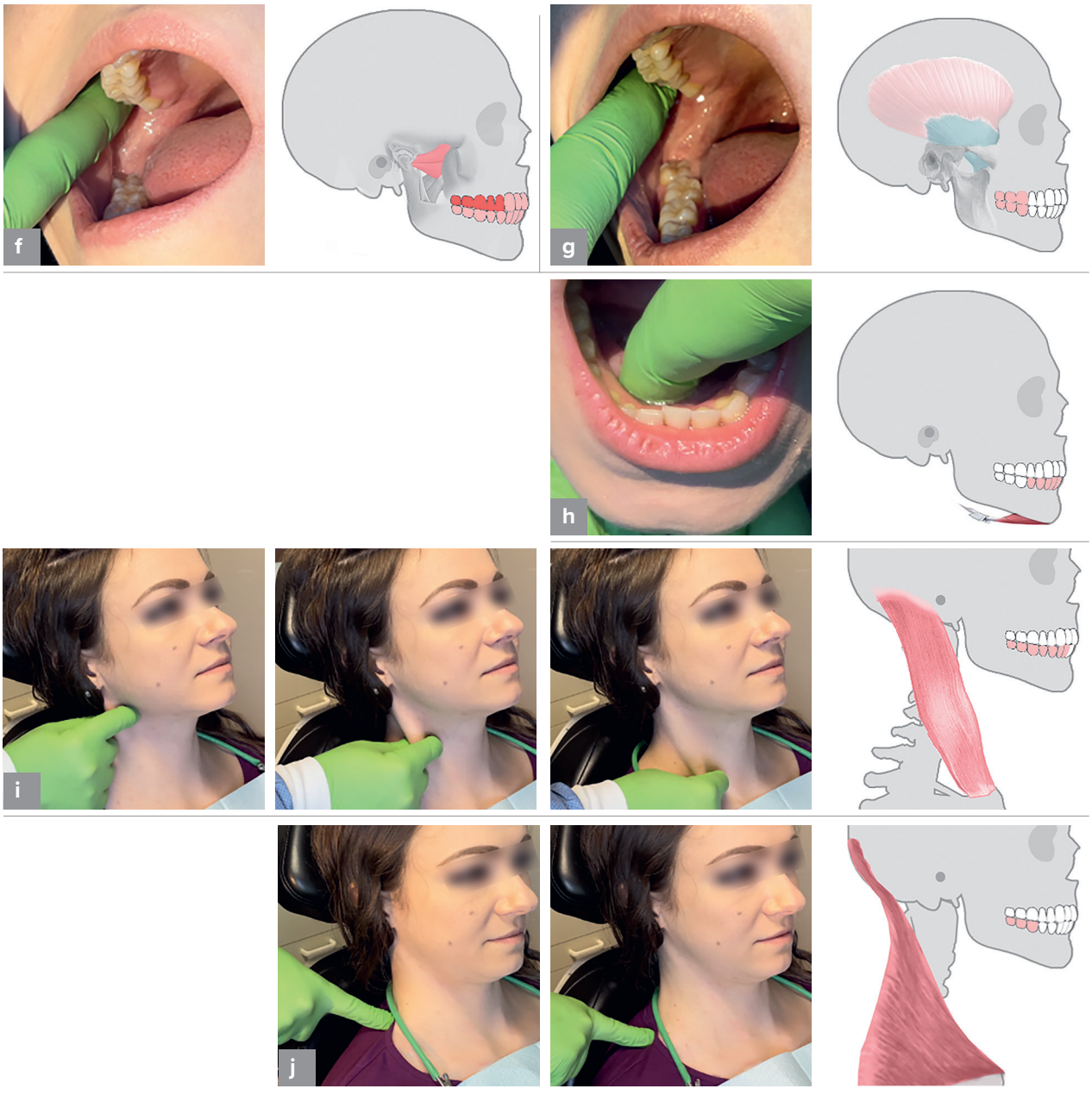


▲ **FIG 15-7** *(cont)* *(f)* Lateral pterygoid muscle. *(g)* Temporalis tendon. *(h)* Anterior digastric muscle. *(i)* Sternocleidomastoid muscle. *(j)* Upper trapezius muscle.

that the patient has painful TMD and there is no obvious endodontic pathosis, invasive dental treatments should be avoided and TMD should be addressed first (Pigg et al 2021).

Additional diagnostic tests may be used if the pain cannot be reproduced with DC/TMD examination, such as a static test (having the patient clench their teeth for 1 minute), dynamic tests (opening, closing, lateral excursions, protrusion, and retrusion are resisted by the examiner), as well as trigger point injections.

Similar to how the endodontic community manages comorbid endodontic-periodontal lesions, a strategy of a combined approach applies to the management of comorbid endodontic/TMD pain. If comorbid myofascial and endodontic pains are diagnosed, the clinician is encouraged to treat the endodontic pain and prescribe the TMD self-care regimen as outlined in Appendix 1: Self-Care Regimen to Ease TMDs and Appendix 2: Exercises to Ease TMDs (included at the end of this chapter) and/or refer the patient to an orofacial pain specialist to manage the TMD pain component.

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If toothache referred from a TMJ or a myofascial structure is suspected to be the sole cause of the patient's pain, it would be prudent for the practitioner to explain the suspected underlying etiology of the pain to the patient, reassure the patient, and prescribe a self-care regimen (see Appendices 1 and 2) for at least 4 to 6 weeks. If the clinician suspects a high level of psychosocial distress and/or multiple overlapping pain conditions (eg, comorbid headaches, fibromyalgia, irritable bowel syndrome, autoimmune conditions, etc), an additional referral to an orofacial pain specialist may also be considered. After that time, the clinician is encouraged to reevaluate the patient to determine if there is a residual odontogenic component or if the patient's pain was strictly musculoskeletal in origin. Once again, it is important to remember that tenderness to percussion alone (without other clinical signs of pulpal-periapical disease and in the absence of identifiable endodontic etiology) should not be interpreted as a sign of endodontic pathosis.

Comprehensive treatment of TMD is largely conservative and includes self-care management, physical therapy, behavioral and stress management, full-coverage occlusal appliances, pharmacotherapy, trigger point injections, and acupuncture (De Leeuw et al, List and Axelsson). Occlusal therapy should be avoided (Al-Ani, Delgado-Delgado et al, Kakudate et al, Li and Leung, Manfredini et al 2017, Management of Temporomandibular Disorders). Surgery is very rarely needed for mechanical TMJ problems and is usually minimally invasive (eg, arthroscopy, arthrocentesis).

Patients need to be educated that TMD treatment is not a quick fix but rather a long-term management, like that of cardiovascular disease or diabetes. Compliance with treatment is of paramount importance to ensure recovery. The home care regimen consists of applying regular hot and/or ice compresses to the jaw; reducing stress and strain on the jaw joint and muscles by keeping the jaw in a restful position where the tongue gently rests on the roof of the mouth and teeth are separated; identifying and avoiding oral habits; staying on a pain-free diet; avoiding gum chewing; keeping good jaw and overall posture; avoiding wide/prolonged mouth opening; reducing intake of caffeine; and maintaining sleep hygiene (see Appendix 1).

TMD-specific physical therapy is beneficial and involves education on jaw exercises, muscle stretching and cross-fiber massage, dry needling, soft tissue mobilization, temporalis tendon icing, iontophoresis, and other modalities. Occlusal appliances should be worn only at night; in most cases of myofascial pain, full-coverage, rigid, flat plane appliances are recommended, which need to be periodically checked and adjusted to ensure that they are balanced.

Pharmacotherapy for TMD typically includes nonsteroidal anti-inflammatory drugs (NSAIDs), muscle relaxants (such as cyclobenzaprine 5 mg at bedtime for 2 to 4 weeks), and occasionally oral steroids, serotonin-norepinephrine reuptake inhibitors (eg, duloxetine, venlafaxine), or sedative agents (eg, hydroxyzine). Treatment may also be supplemented with trigger point injections and acupuncture. Trigger point injections should be performed by preparing the injection site with an alcohol wipe, palpating the muscle to identify the taut muscle band, stabilizing it between the fingers, inserting a 25- to 27-gauge needle into the muscle, aspirating to prevent intravascular injection, and administering 0.5 to 1 mL of 0.5% bupivacaine or 1% lidocaine without epinephrine. The effect of the injections may be augmented if they are followed by a spray-and-stretch technique, whereby a vapocoolant spray is administered over the muscle in the direction of the muscle fibers three to four times while asking the patient to slowly stretch their mouth.

Stress management is important to reduce tension in the muscles. Patients may learn stress-coping strategies on their own or benefit from a referral to a pain psychologist who can assist the patient in learning relaxation techniques, developing appropriate pain-coping strategies, complying with treatment, as well as managing mood disorders and autonomic regulation through biofeedback tools, cognitive behavioral therapy, mindfulness/meditation, eye movement desensitization and reprocessing therapy, clinical hypnosis, etc. Finally, referral and management of other comorbid chronic pain conditions may be necessary.

## Nonodontogenic Toothache of Sinus or Nasal Mucosal Origin

Dental pain is a possible symptom of acute maxillary sinusitis, which typically develops after an upper respiratory tract infection commonly caused by a viral infection. Chronic sinusitis is rarely painful, and it is unlikely for dentists to encounter patients experiencing tooth pain due to chronic sinus disease. In most cases, inflammatory paranasal sinus disease that leads to facial pain occurs within a week following an upper respiratory tract infection (Bell et al). Symptoms of acute rhinosinusitis commonly include nasal congestion, rhinorrhea, purulent nasal discharge, postnasal drip, facial pressure and pain, changes in sense of smell, cough, fever, pharyngitis, halitosis, headache, earache, and toothache (Lanza and Kennedy).

A study assessing the clinical presentation of acute and subacute paranasal sinusitis found that 11% of patients had maxillary tooth pain (Williams et al). The pain is often described as pressure, fullness, dull, aching, or throbbing. Dental examination findings include sensitivity to percussion, palpation, and chewing in multiple maxillary


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posterior teeth as well as a generalized hyperresponse to pulp cold testing of the vital teeth in the absence of an obvious dental etiology.

A diagnosis of sinogenic toothache is made based on a combination of the following (Bell et al):

1. A history of current or recent upper respiratory tract infection, unilateral nasal obstruction and discharge, and impaired sense of smell
2. Clinical findings of increased pain on vertical change in head position (patient may be asked to dip the head below the knees while sitting) and pain to percussion or pressure over the sinuses (and in the ENT setting, observation of pus in middle meatus)
3. Radiographic signs of mucosal thickening of at least 6 mm or air-fluid level

Noteworthy is that pain arising from tension headaches, migraines, atypical facial pain, or TMD is frequently misattributed to sinusitis because patients often localize the pain to the sinonasal region (Jones).

The primary approach to treating acute sinusitis focuses on symptom relief and does not typically involve antibiotics unless the patient has a fever or there are signs of infection spreading beyond the sinus area (Chan and Kuhn). Instead, treatment primarily involves using topical nasal decongestants and saline irrigation of the nasal cavity (Bell et al). It is important to limit the use of nasal decongestants to no more than 7 days to avoid rebound mucosal swelling (Graf). Saline irrigation of the nasal cavity helps clear surface debris and promotes sinus drainage (Harvey et al).

## Nonodontogenic Toothache of Neurovascular or Headache Origin

Headaches have been shown to present with pain in the orofacial region in the V2 or V3 distributions of the trigeminal nerve, but they are often misdiagnosed because they closely resemble sinus pain or toothache (Alonso and Nixdorf, Groenke et al, Hryvenko et al, Sharav et al 2017). The most prominent representatives of this group of primary headaches are migraine, tension-type headache, and autonomic cephalalgias such as paroxysmal hemicrania, hemicrania continua, cluster headache, and short-lasting unilateral neuralgiform headache attacks (SUNHA). The ICOP classifies these as a category of "orofacial pains resembling presentations of primary headache" that includes four main conditions—orofacial migraine, tension-type orofacial pain, trigeminal autonomic orofacial pain, and neurovascular orofacial pain—and provides diagnostic criteria for each (ICOP).

Several case series and case reports have previously described primary headaches presenting in the face and teeth (Alonso and Nixdorf, Groenke et al, Hryvenko et al). A recent large cohort of nearly 3,000 headache patients in a tertiary care center found V2 and/or V3 facial pain involvement in 2.3% of migraine patients, 14.8% of cluster headache patients, 45% of patients with paroxysmal hemicrania, 21.4% of hemicrania continua patients, and 20% of SUNHA patients (Ziegeler and May). Another study of 517 people found that, in addition to head pain, 8.9% of migraine patients had pain in the lower half of the face (Yoon et al). Up to half of migraine patients will be evaluated by a dentist, and 20% to 65% will undergo unnecessary dental treatments (Bahra and Goadsby, Hryvenko et al, Lambru et al 2020, Rossi et al).

In clinical practice, three types of patients can be seen with orofacial pains resembling presentations of primary headache (ICOP):

- **Type I:** Headache with additional facial pain
- **Type II:** Headache stopped and was replaced by facial pain of similar presentation
- **Type III:** De novo facial pain resembling primary headache without preceding headache history

Orofacial migraine (OFM) and neurovascular orofacial pain (NVOP) have many features in common (Sharav et al 2023). OFM and NVOP are classified under the type III category, meaning that such patients experience pain exclusively in the face without head pain or history thereof. Migraine pain presenting in the midface or lower half of the face without affecting the head is rarer but not uncommon (Benoliel et al, Eross et al). An estimate of the prevalence of OFM ranges from 5% to 9% of migraine patients, or 1% to 2% of the general population (Daudia and Jones, Lambru et al 2020, Sharav et al 2023, Yoon et al).

Type II and III orofacial pains resembling migraine pose a great diagnostic challenge to general dentists and endodontists. Dental examination findings can further confuse a clinician as teeth may show an exaggerated response to cold testing and percussion secondary to allodynia and sensory hypersensitivity (light, noise, pressure, movement) seen in migraine. A recent study showed that up to 65% of patients diagnosed with OFM have undergone root canal treatments and extractions with no relief (Lambru et al 2020). Obtaining a detailed, thorough pain history is therefore key to preventing misdiagnosis of pain as odontogenic and inappropriate dental treatments.

Type II and III orofacial pain resembling migraine typically presents in the unilateral maxillary posterior teeth as pulsating pain of moderate to severe intensity, mimicking symptoms of symptomatic irreversible pulpitis. The pain is recurrent and can vary in duration from several hours

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to several days at a time. Physical activity, stress, lights, and noises tend to aggravate it, while laying down in a dark quiet room helps with the pain. Pain may be associated with nausea and/or vomiting (ICOP). With type II presentation, a detailed history will usually reveal that these patients had a headache that changed around the time the tooth pain started. Usually, these patients take multiple and frequent over-the-counter and prescription pain medications for pain relief. Thorough discussion of the patient's medical, psychosocial, and sleep history may show that they suffer from other chronic pain conditions (TMD, fibromyalgia), anxiety, and depression and have a family history of headaches. Further clinical interview may uncover that pain is associated with typical migraine triggers, such as stress, lack of sleep/oversleeping, delayed or skipped meals, caffeine, chocolate, aged cheese, cured meats, red wine, artificial sweeteners, monosodium glutamate (MSG), and barometric pressure changes.

Referral to an orofacial pain specialist or a headache neurologist is appropriate, and the migraine treatment includes avoidance of migraine triggers (keeping a headache journal can be used to help identify pain triggers); management of stress and mood disorders; abortive migraine medications (such as sumatriptan); or preventive pharmacotherapy with either beta-blockers (eg, propranolol), anticonvulsants (eg, topiramate), calcitonin gene-related peptide monoclonal antibodies, botulinum toxin injections according to the FDA-approved protocol for chronic migraine prevention, sphenopalatine ganglion blocks, or external trigeminal nerve stimulation. Further, migraine is considered to be one of the chronic overlapping pain conditions (Maixner et al, Slade et al). Migraine is also a known risk factor for the onset of TMD (Tchivileva et al 2017), and migraine-TMD comorbidity is very common (Dahan et al, Tchivileva et al 2021). Therefore, identifying and addressing other comorbid chronic pain conditions in migraine patients via an interdisciplinary approach is advised to improve treatment success.

NVOP and trigeminal autonomic orofacial pain are more rare pain conditions. NVOP presents very similar to OFM, with unilateral, moderate to severe, pulsating or toothache-like pain that may be shorter in duration (1 to 4 hours) and is accompanied by one of the following: ipsilateral lacrimation and/or conjunctival injection, ipsilateral rhinorrhea and/or nasal congestion, ipsilateral cheek swelling, photophobia and/or phonophobia, nausea and/or vomiting (ICOP). Trigeminal autonomic orofacial pain exclusively presents in the orofacial region but is associated with features of trigeminal autonomic cephalalgias. This category includes orofacial cluster attacks, paroxysmal hemifacial pain, short-lasting unilateral neuralgiform facial pain attacks with cranial autonomic symptoms, and hemifacial continuous pain with autonomic symptoms (ICOP). Details regarding diagnosis and management of these more rare conditions can be found elsewhere (ICOP).

## Nonodontogenic Toothache of Neuropathic Origin

The neuropathic pain cluster can be divided into episodic and continuous neuropathic pains.

### Episodic neuropathic toothache

#### Trigeminal neuralgia

A classic example of episodic neuropathic pain is trigeminal neuralgia (TN). TN is a very rare condition with a prevalence of 0.03% to 0.3%, an incidence of 4 to 8 people per 100,000 per year, and a peak incidence at age 50 to 60 years (De Toledo et al, Koopman et al, Lambru et al 2021, Maarbjerg and Benoliel, van Hecke et al, Zakrzewska 2003, Zakrzewska and Linskey). A fourth of TN patients consult dentists regarding their pain, and 33% to 65% of them receive unwarranted dental treatments (Sharav and Benoliel, Truelove, von Eckardstein et al).

TN pain is characterized by sudden onset with no precipitating event. The pain never crosses the midline and follows the distribution of one or more branches of the trigeminal nerve. Pain along the distribution of one branch is the most common and occurs in about 40% of TN cases; however, two and even three branches could be involved (Bennetto et al, Sharav and Benoliel). The pain is shooting, electric shock–like, stabbing, or sharp (ICOP) and of such severe intensity that the patient may admit to contemplating suicide. It can be provoked by talking (76%), chewing (74%), touch (65%), temperature (cold 48%, heat 1%), wind, or shaving (Maarbjerg et al, Sharav and Benoliel). The pain lasts from a fraction of a second to 2 minutes, but sometimes it can be accompanied by continuous dull, throbbing, or burning pain (Bennetto et al, ICOP, Lambru et al 2021, Maarbjerg and Benoliel, Maarbjerg et al, Zakrzewska and Linskey). Pain attacks can be accompanied by classic contraction of the facial musculature, which is why TN has also been called *tic douloureux*. Some patients may also have mild autonomic signs associated with the attacks. Pain remission has been reported and may last from several weeks to several years, with the second attack occurring within 5 years (65%) or 10 years (77%) of the first one (Sharav and Benoliel, Zakrzewska and Linskey).

Comorbidity of TN with cluster headache (Alberca and Ochoa), chronic paroxysmal hemicrania (Hannerz), hemicrania continua (Hryvenko et al), and glossopharyngeal neuralgia (Rushton et al) has been reported. Endodontic


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and TMD pain may also co-occur with TN, and the clinician might need to tease the symptoms of these conditions apart to establish a complete and correct diagnosis. Further, the presentation of short-lasting, unilateral, neuralgiform headache attacks with conjunctival injection and tearing (SUNCT) is very similar to TN with autonomic signs and should be considered in the differential diagnosis, as these conditions respond to different treatments (Lambru and Matharu).

TN is often attributed to a vascular compression of the trigeminal nerve root, but sometimes no compression can be found. It can also be secondary to multiple sclerosis or a space-occupying lesion (Bennetto et al, ICOP, Lambru et al 2021, Maarbjerg and Benoliel, Sharav and Benoliel, Zakrzewska and Linskey). A referral to an orofacial pain or neurology clinic is therefore indicated for further evaluation, and a brain MRI must be taken to exclude the secondary causes.

Treatment of TN is divided into pharmacologic or surgical options. Effective pharmacotherapy is achieved with oxcarbazepine or carbamazepine, with or without an add-on baclofen, lamotrigine, gabapentin, or pregabalin (Bendtsen et al, Lambru et al 2021). However, these drugs cause significant side effects and require monitoring of blood counts as well as liver and kidney function. Botulinum toxin injections have also shown to be promising, with fewer side effects (Rubis and Juodzbalys, Wu et al, Zhang et al). Brain surgery is also successful and could be *(1)* nerve sparing (microvascular decompression), *(2)* nerve destructive (radiofrequency, glycerol, or balloon percutaneous rhizotomy), or *(3)* radiosurgery (gamma and cyber knife) (Lambru et al 2021).

#### Pretrigeminal neuralgia

*Pretrigeminal neuralgia* (PTN) refers to prodromal or early symptoms that precede the onset of typical TN. Recognition of this early form of TN is very challenging because the pain presentation is vaguer, and clear and consistent diagnostic criteria are lacking. Patients with PTN describe their pain as similar to a toothache or sinusitis. PTN presents as a dull, continuous pain (Maarbjerg and Benoliel) lasting for several hours and occasionally triggered by jaw movements or the consumption of hot or cold beverages (Fromm et al), which mimics pulpal pathosis. PTN affects the same division of the trigeminal nerve where the typical TN pain attacks later develop (Fromm et al). Classical TN manifests from several days to as long as 12 years after the initial onset of this prodromal pain (Fromm et al). PTN is a diagnosis of exclusion and is usually made in retrospect after classic TN develops. Early diagnosis may be established by a response to oxcarbazepine or carbamazepine (Fromm et al).

#### Glossopharyngeal neuralgia

Glossopharyngeal neuralgia (GN) has similar characteristics to TN but presents in the distribution of the glossopharyngeal nerve. It is even rarer than TN, with an incidence of 0.7/100,000 people per year (Manzoni and Torelli). Unlike TN, GN pain is felt in the ear, base of the tongue, tonsillar fossa, or beneath the angle of the jaw and is provoked by swallowing, chewing, talking, or yawning. MRI scan is required to rule out GN secondary to other pathology. The treatment of GN is similar to that of TN (Kondo, Rozen, Stieber et al, Zakrzewska and Patsalos).

## Continuous neuropathic toothache

This category has historically included a persistent pain condition following invasive dental procedures and has been known as *phantom tooth pain*, *atypical odontalgia*, *persistent dentoalveolar pain disorder*, and *painful posttraumatic trigeminal neuropathy*. These terms and the respective diagnostic criteria, which were based on different expert opinions and not evidence-based classification systems, have been commonly used to describe the same phenotype, with presumed underlying neuropathic pain mechanisms. Most recently, experts from around the world revised the diagnostic criteria and proposed two separate terms for conditions that had been commonly described and researched as one: *post-traumatic trigeminal neuropathic pain* (PTTNP) and *persistent idiopathic dentoalveolar pain* (PIDAP) (ICOP). There are indeed significant similarities between PTTNP and PIDAP, as both disorders are associated with similar symptoms, long pain duration, care seeking, and insufficient pain relief (Pigg et al 2013). ICOP differentiates the two based on what we know of the pathophysiology of pain (neuropathic vs idiopathic), temporal relationship with the injury (within 6 months of the injury vs no clear preceding causative event), and the location of the somatosensory changes (in a neuroanatomically plausible distribution of CN V vs not limited to a neuroanatomically confined area) (ICOP). What differentiates PTTNP from PIDAP most clearly is not only the history of nerve injury but also diagnostic test confirmation of a lesion of the peripheral trigeminal nerve(s) explaining the pain (ICOP).

#### Post-traumatic trigeminal neuropathic pain

*Post-traumatic trigeminal neuropathic pain* (PTTNP) is defined as unilateral or bilateral facial or oral pain that occurs after accidental or surgical injury of the peripheral branches of the trigeminal nerve, persists or recurs for more than 3 months, and is accompanied by symptoms and/or clinical signs of trigeminal nerve dysfunction (ICOP). Some examples of the events causing nerve injury that are encountered in the dentistry setting are

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nonsurgical and surgical root canal treatments, implants, anesthetic injections, and extractions. The mechanism after root canal treatment is thought to be a deafferentation of nociceptive primary afferents in the tooth pulp.

PTTNP is a very rare condition. Until the ICOP was published in 2020, PTTNP and PIDAP were commonly referred to and studied as the same condition, and their combined prevalence after root canal treatment has been estimated to range from 0.3% to 1.6% (Nixdorf and Moana-Filho, Nixdorf et al 2015, Ram et al), meaning that PTTNP in its stricter definition is probably even rarer than that. Interestingly, in a study of more than 1,900 patients, incidence of neuropathic pain after surgical extraction of impacted third molars, where tissue injury is expected to be more extensive than in root canal treatment, was found to be 0%, and the vast majority of the persistent pain cases were found to be of TMD origin (Berge). After dental implant surgery or a sagittal split ramus osteotomy, the estimated incidence of neuropathic pain is 0.1% to 0.5% (Politis et al 2017, Marchiori et al, Politis et al 2014), where the more common sequela of the nerve injury is paresthesia (Ardekian and Dodson, Renton et al).

Therefore, when assessing persistent pain after root canal treatment, it is important to first rule out other more common nonodontogenic diagnoses as the etiology of pain before considering neuropathic pain in the differential diagnosis. It is prudent to differentiate between "persistent pain" (a symptom) and "neuropathic pain" (a diagnosis) and not to lump them together without a thorough history, examination, and exclusion of other pain causes, something several published studies have unfortunately done over the years, thereby creating a perception of an exaggerated prevalence of neuropathic pain in endodontic settings. Such mislabeling may lead to the actual cause of pain remaining unaddressed and further chronification of persistent pain.

The clinician must conduct a comprehensive evaluation of the patient's pain history. Patients with PTTNP report history of a trauma to the jaw/teeth or invasive dental procedure(s) with a potential for damage to the peripheral nerve branches. It is important to note that, for the diagnosis of PTTNP, pain onset must have started within 6 months *after* the dental treatment/trauma, not before it. Pain is unilateral, within the dermatome of the affected nerve, and doesn't cross the midline. Pain intensity varies from moderate to severe (5 to 8/10). Patients describe their pain as dull, deep, burning, shooting, as well as squeezing, prickling, or itching sensations. Dysesthesia and numbness can also be associated with the pain. Another important point is that the pain is continuous—it does not come and go—although it varies in intensity throughout the day. Neuropathic pain features also include lack of response to anti-inflammatory medications, improved symptoms in the morning, and worsening of pain with stress, fatigue, or illness. Besides the unusual pain characteristics, the rest of the symptoms often resemble inflammatory tooth pain and are interpreted as remaining root canal infection (Pigg et al 2021). Patients may also complain of swelling, foreign body sensation, or hot or cold feeling in the area. These patients also seek care and may receive multiple pharmacologic and dental treatments with little to no relief (Pigg et al 2013).

A clinical assessment of PTTNP should include the following and should be compared to the responses on the contralateral side:

1. Basic mechanosensory examination, recording whether there is hyper- or hypoesthesia to the following:
   - Light touch (showing allodynia, anesthesia, or paresthesia)
   - Pinprick with sharp side of the explorer (hyperalgesia, hypoalgesia)
   - Thermal stimulus with a cold spatula (allodynia, anesthesia/paresthesia)
2. Visual inspection and palpation to examine for any autonomic changes in color, temperature, sweating, or swelling of the painful tissues

On clinical examination, despite abnormal somatosensory findings, tissues appear healed from the previous injury. On radiographic examination, there are no signs of periapical pathosis.

To establish PTTNP as a diagnosis, the following diagnostic criteria must be met (ICOP):

A. Pain in a neuroanatomically plausible area within the distribution(s) of one or both trigeminal nerve(s), persisting or recurring for > 3 months and fulfilling criteria C and D
B. Both of the following:
   1. History of a mechanical, thermal, radiation, or chemical injury to the peripheral trigeminal nerve(s)
   2. Diagnostic test confirmation of a lesion of the peripheral trigeminal nerve(s) explaining the pain
C. Onset within 6 months after the injury
D. Associated with somatosensory symptoms and/or signs in the same neuroanatomically plausible distribution
E. Not better accounted for by another ICOP or *International Classification of Headache Disorders* (ICHD-3) diagnosis (ICOP)

Only about a third of patients with this neuropathic pain improve over 7 to 19 years, while almost half continue to experience the same or worse pain for many years


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after onset (Allerbring and Haegerstam, Pigg et al 2013). Complete remission can be expected in less than one in four patients (Pigg et al 2013). In contrast, almost 50% of patients with TMD pain experience complete pain resolution within 5 years (Ohrbach and Dworkin).

A majority of patients with such neuropathic pain continue to seek and receive dental treatments (most commonly, endodontic treatment and extractions) with no relief (Ram et al). It is important to remember that the invasive treatment directed to the teeth will not improve the pain. On the contrary, the pain may increase after endodontic retreatment, apical surgery, or extraction due to reinjury of the nerve branches (Pigg et al 2021).

Only in cases of an acute, clear-cut macroscopic nerve injury from the apical surgery, implant placement, or extraction is an urgent surgery (within 30 hours of the injury and rarely up to 3 months) (Renton and Yilmaz) indicated to repair the sectioned nerve and to remove the implant or other foreign body impinging on the nerve; this should be supplemented with a corticoid taper for 5 to 7 days followed by 3 weeks of NSAIDs.

In cases of delayed presentation, the treatment consists of topical and systemic pharmacotherapy. Systemic pharmacotherapy includes tricyclic antidepressants (eg, amitriptyline), serotonin-norepinephrine reuptake inhibitors (eg, duloxetine), and anticonvulsants (eg, gabapentin, pregabalin). Given the significant side effects of the systemic medications, patients are usually started on topical medications used in the custom-made intraoral tissue stent: benzocaine 20% in Orabase; off-label compound medication with 2% lidocaine, 2% tetracaine, and 10% gabapentin in Orahesive; clonidine 0.01% in PLO base; or ketamine 0.5% in PLO base or in Orabase (Coulter and Nixdorf, Padilla et al). Botulinum toxin injections for management of PTTNP are also promising and have been shown to produce significant pain reduction with few side effects (Capon et al, Cuadrado et al, Garcia-Saez et al, Babiloni et al 2016). Psychosocial therapy can be beneficial and is aimed at improving the patient's ability to cope and manage the pain and reduce the impact of the pain on their quality of life.

#### Other trigeminal neuropathic pain

This category refers to facial pain experienced in the area supplied by one or more branches of the trigeminal nerve, caused by an underlying disorder, and indicates nerve damage (ICOP). The pain is typically continuous or nearly continuous and is often described as burning, squeezing, aching, or like pins and needles. While brief episodes of pain may occur intermittently, they are not the predominant type of pain in this category.

#### Trigeminal neuropathic pain attributed to herpes zoster

Chickenpox is a result of varicella zoster virus infection. After the initial infection, the virus can lie dormant in the sensory nerve cell bodies and potentially reactivate years later, causing a condition known as *herpes zoster* or *shingles*. It predominantly affects the elderly and immunocompromised individuals. Typical symptoms of herpes zoster include itching, numbness, or tingling in the affected area, followed by the eruption of vesicles and development of pain. The incidence of herpes zoster is approximately 0.4% per year in the US population (US Centers for Disease Control and Prevention Clinical Overview of Shingles). The disease typically affects thoracic and lumbar regions. In approximately 10% to 15% of cases, herpes zoster affects the trigeminal ganglion, with the ophthalmic division being predominantly involved in about 80% of such patients (ICOP). Up to 25% of patients are pain free (Dworkin et al). Thus, an estimated frequency of herpes zoster causing pain in the V2 and V3 divisions of the trigeminal nerve, with or (more likely) without involvement of teeth, is approximately 0.008%. Therefore, it is exceptionally rare to encounter herpes zoster as an etiology of tooth pain. There have been only a handful of documented cases of herpes zoster causing nonodontogenic toothache, occurring in the prodromal stage of the disease before the appearance of blisters. These reported cases show varying presentations, with some describing symptoms resembling pulpal pain and others indicating pulp necrosis with periapical lesions (Fristad et al, Goon and Jacobsen, Gregory et al, Lopes et al, Paquin et al). Diagnosis of toothache attributed to herpes zoster is usually made in retrospect after the eruption of vesicles.

Trigeminal neuropathic pain attributed to herpes zoster is described in ICOP as a unilateral facial pain lasting less than 3 months, localized in the distribution of one or more branches of the trigeminal nerve, and attributed to and accompanied by symptoms and/or clinical signs of acute herpes zoster: burning, stabbing, shooting, tingling, or aching pain accompanied by cutaneous allodynia. It may be associated with third, fourth, and sixth cranial nerve palsies. On examination, herpetic eruption is present in the same trigeminal distribution. When the diagnosis is suspected but vesicles do not appear, polymerase chain reaction testing may be performed to detect varicella zoster virus in the cerebrospinal fluid (ICOP).

Management of herpes zoster is pharmacologic with a 7-day course of antivirals such as valacyclovir and famciclovir. Recombinant zoster vaccine is recommended to prevent herpes zoster infection and its complications.

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## Nociplastic Pain

Nociplastic pain is a mechanistic category of pain distinct from nociceptive pain (caused by ongoing inflammation or tissue damage) and neuropathic pain (caused by nerve damage). The International Association for the Study of Pain (IASP) defines *nociplastic pain* as "pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion of the
somatosensory system causing the pain" (International Association for the Study of Pain, Kosek et al).

The precise mechanisms behind this type of pain are not completely understood, but it is believed that heightened CNS pain and sensory processing, along with changes in pain modulation, are significant factors. Nociplastic pain is characterized by a variety of symptoms that go beyond what would be expected based on the level of tissue or nerve damage. These symptoms can include more widespread or severe pain and additional CNS-related issues like fatigue, sleep disturbances, memory problems, and mood changes. Nociplastic pain can manifest either on its own, as seen in conditions like fibromyalgia or tension-type headaches, or in combination with ongoing nociceptive or neuropathic pain, such as in chronic low back pain. Recognizing this type of pain is crucial because it requires different treatment approaches compared to nociceptive pain. It tends to have reduced responsiveness to therapies targeting the peripheral system, such as anti-inflammatory drugs, opioids, surgery, or injections (Fitzcharles et al).

## Persistent idiopathic dentoalveolar pain

PIDAP is a "persistent unilateral intraoral dentoalveolar pain, rarely occurring in multiple sites, with variable features but recurring daily for more than 2 hours per day for more than 3 months, in the absence of any preceding causative event" (ICOP).

Prior to the ICOP 2020 classification of orofacial pain conditions, PIDAP was called *PDAP*, *atypical odontalgia*, or *phantom tooth pain*. Given that the term *persistent idiopathic dentoalveolar pain* (PIDAP) is relatively new, previous studies used earlier terminology, and a substantial portion of the existing evidence is inherently linked to PTTNP; this makes estimating the prevalence of PIDAP a challenge. It has been documented to reach rates as high as 2.1% (Ram et al); however, this specific sample was derived from a university-based orofacial pain center, which may limit its generalizability to the broader population.

Patients may present with the following symptoms, which should prompt clinicians to consider the possibility of PIDAP (Coulter and Nixdorf, Nixdorf and Moana-Filho): difficulty localizing pain, which can affect multiple teeth; continuous pain that does not disrupt sleep; pain described as a burning or electric shock–like; presence of numbness or tingling sensations; a history of pain that has persisted despite multiple attempts to alleviate it with irreversible treatments; and pain that has persisted beyond the expected healing time frame. Patients with PIDAP may also report a history of previous trauma to the affected area but no signs of local cause can be identified (which contrasts with PTTNP, which requires the presence of a peripheral trigeminal nerve lesion for diagnosis). Clinically, teeth may respond abnormally to tooth percussion and sensibility testing, but there is no apparent evidence of local pathology (Coulter and Nixdorf). The pain shows limited to no response to local anesthetics (Finnerup et al, List et al 2007).

The diagnostic criteria for PIDAP according to ICOP 2020 are summarized as follows (ICOP):

1. History
   - *Location:* Intraoral pain localized to a dentoalveolar site or sites, such as a tooth or alveolar bone; typically, pain is unilateral and rarely in multiple sites, but with time, it may spread to a wider area of the craniocervical region
   - *Timing:* Recurring daily for > 2 hours per day for > 3 months
   - *Quality:* Deep, dull, pressure-like, although a wide variety of words are used to describe the character and quality of the pain
   - *Aggravating factors:* The pain can have exacerbations and be aggravated by stress
2. Clinical and radiographic examination
   - Normal
   - Somatosensory testing with pinprick or light touch only very rarely shows sensory abnormalities
   - Local causes have been excluded
3. Not better accounted for by another ICOP or ICHD-3 diagnosis

It is important to recognize that PIDAP is a diagnosis of exclusion. Further, PIDAP may present as an isolated condition, but it commonly occurs as part of a mixed pain in combination with TMD pain, tension-type headache, and widespread pain (List et al 2007). This may be due to central sensitization and somatosensory abnormalities, which were observed in 85% of patients with PIDAP (List et al 2008). PIDAP has been proposed as one of the chronic overlapping pain conditions, along with conditions such as TMDs, fibromyalgia, irritable bowel syndrome, chronic fatigue syndrome, chronic tension-type headache, migraine, chronic lower back pain, etc (Babiloni et al 2020).


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Similar to PTTNP, a majority of PIDAP patients suffer from pain persisting for many years with little to no relief or even amelioration of pain despite many attempted treatments (Pigg et al 2013). The patient needs to be educated that their pain is not of dental origin and therefore is unlikely to improve through dental treatments. Because the etiology of PIDAP is poorly understood, management is directed to reduce the symptoms of pain and follows the principles of treatment of PTTNP, such as systemic and topical pharmacotherapy and psychosocial therapy (see previous section). Considering a role of central sensitization in the pathophysiology of PIDAP, a patient's comorbid chronic pain conditions, especially in the trigeminal innervation territory (such as TMD, migraine, tension-type headache), also need to be managed to minimize the burden of additional continuous nociceptive input into the CNS. In addition, about half of patients with PIDAP also have comorbid psychiatric disorders, and the emotional aspect of pain may be more prominent than the sensory one in such patients (Miura et al). Multidisciplinary and holistic approaches to treatment are therefore indicated.

## Nonodontogenic Toothache of Cardiac Origin

Dentists must be mindful of cardiac pain manifesting as jaw and tooth pain, as this can be a life-threatening condition and require immediate medical attention. In some cases, patients may only report dental symptoms.

While a study conducted in an emergency care unit did not report orofacial pain as the sole symptom of a heart attack (Then et al), other studies found that throat pain and orofacial pain were present in 34% to 38% of patients with cardiac ischemia (Kreiner et al 2020). The affected orofacial areas included the upper throat (82%), left mandible (45%), right mandible (41%), and left TMJ and ear (18%). In one study, cardiogenic toothache was present in three patients (4%), affecting the bilateral mandibular teeth in two patients and the left maxillary teeth in one patient (Kreiner et al 2007).

The nature of cardiogenic pain is typically described as pressing or burning (De Laat) and less commonly as deep, diffuse, or pulsating (Okeson 2012). Physical exercise can often trigger or aggravate the pain (De Laat). The pain may be associated with chest pain, neck pain, throat pain, shoulder pain, a history of cardiovascular disease, and relief with nitroglycerin tablets (Hargreaves et al). If the patient's medical history aligns with the absence of an obvious dental etiology, immediate referral to a cardiologist is crucial (De Laat).

## Nonodontogenic Toothache of Psychogenic Origin

The interplay of psychologic factors and chronic pain has been widely accepted and extensively studied within the context of the biopsychosocial model. Depression, anxiety, and stress influence the development and perpetuation of chronic pain; however, it is extremely rare for pain to be solely psychogenic in origin. While there are no known published reports of toothache of psychogenic origin, some known psychologic disorders have a potential to be an etiology of dental pain, including somatic symptom disorder, illness anxiety disorder, conversion disorder, and factitious disorder (American Psychiatric Association).

Patients with somatic symptom disorder may report physical symptoms that are dramatic, vague, and exaggerated but not intentionally simulated or falsified. These symptoms cause the patient significant distress disproportionate to the actual severity of those symptoms, causing them to dedicate a considerable amount of time to seeking medical or dental care. They may report having a complex medical history with many physical diagnoses having been considered to explain their symptoms, which typically start before the age of 30 years. Despite the effort such individuals put in seeking evaluations, diagnostic tests, medication trials, and surgeries, they are usually not compliant with treatment and often suffer iatrogenic complications (De Leeuw et al).

The clinical characteristics of toothache of somatoform origin are thought to include the following (Hargreaves et al):

- Pain in multiple teeth, which may shift from one tooth to another or to different locations
- Toothache is chronic and does not significantly change over time
- Pain deviates from normal or expected patterns
- Pain does not respond or shows unexpected response to dental treatments
- The patient exhibits frequent health care utilization, excessive reliance on others, a withdrawn or nonfunctional lifestyle, or significant use of medications
- The source of pain cannot be identified, and the clinical characteristics do not align with any other known pain condition

Conversion disorder is a condition in which an individual experiences symptoms resembling those of a neurologic disorder, such as paralysis, weakness, abnormal movements, sensory disturbances, or seizures, but they are not due to any known medical condition and instead are believed to be a manifestation of psychologic distress

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or unresolved emotional conflicts. Conversion disorder is thought to involve a disruption in the way the brain processes or expresses emotions, leading to the conversion of emotional distress into physical symptoms.

Factitious disorder, also known as *Munchausen syndrome*, is a psychologic condition in which individuals intentionally fabricate or exaggerate physical or psychologic symptoms to assume the role of a sick person. The motivation behind this behavior is to gain attention, sympathy, and medical care. People with factitious disorder may go to great lengths to deceive medical professionals, undergo unnecessary tests and procedures, and even self-inflict harm to maintain their illness. Unlike malingering, where symptoms are falsified for external gain, factitious disorder is driven by the internal need for a sick role.

These conditions fall under the category of mental disorders and are most effectively addressed by a skilled psychologist or psychiatrist.

## Nonodontogenic Toothache of Systemic Origin

Pulpal, periodontal, and jaw pain may be attributed to various systemic causes. For example, sickle cell anemia can lead to pulpal pain when sickle cells obstruct blood flow to the pulp, causing tissue damage. ICOP diagnostic criteria generally require the systemic disease to be diagnosed, the causation of the pain to be clinically plausible, and the pain to not be better accounted for by another ICOP diagnosis.

It is common for multiple teeth to be impacted when a systemic disease causes pulpal pain. Hematologic and genetic disorders, such as neutropenia, leukemia, Down syndrome, and various syndromes (leukocyte adhesion deficiency, Papillon-Lefèvre syndrome, Chediak-Higashi syndrome, histiocytosis syndromes, glycogen storage disease, infantile genetic agranulocytosis, Cohen syndrome, Ehler-Danlos syndrome types IV and VIII, or hypophosphatasia) can contribute to periodontal pain. Jawbone pain can be associated with sickle cell disease, Gaucher's disease, and Paget's disease, with symptoms ranging from acute episodes to chronic pain and bone changes. Jawbone pain in sickle cell anemia can occur weekly or infrequently, leading to bone infarcts, necrosis, degenerative changes, and chronic pain. Gaucher's disease is a metabolic disorder that causes diffuse bone pain and episodes of osteonecrosis. Paget's disease of bone is characterized by abnormal bone growth, often affecting multiple areas such as the skull, spine, pelvis, and long bones, leading to localized pain and swelling (ICOP).

Jawbone pain can be caused by both benign and malignant lesions. Benign bone tumors are often discovered incidentally and may present with localized pain, swelling, or deformity. Giant cell tumor of bone is a rare, benign neoplasm that typically causes pain and swelling. Osteoid osteoma is characterized by progressively increasing pain relieved by aspirin or NSAIDs. Osteoblastoma causes chronic, continuous pain that is not relieved by medication. Malignant lesions, whether primary or metastatic, can also lead to jawbone pain. Primary malignancies include osteosarcoma, Langerhans cell histiocytosis, non-Hodgkin lymphoma, and multiple myeloma. Osteosarcoma commonly presents with localized pain and is rare in the jawbones. Langerhans cell histiocytosis may cause jaw pain and tooth loss. Non-Hodgkin lymphoma can be challenging to diagnose and may present as unclear dental pain. Multiple myeloma often results in extensive skeletal destruction and bone pain, especially in the back or chest. Remote malignant lesions can cause pain through direct mass effects or paraneoplastic effects without metastasis to the jaw (ICOP).

Toothache caused by neoplasia is uncommon but possible. While certain primary tumors such as Schwannoma, lymphoma, or pontine tumors affecting the trigeminal nerve can be associated with tooth pain, metastatic cancer (particularly breast carcinoma or metastatic renal cancer) has been reported as a potential cause (De Laat). Significant findings that raise suspicion of dental pain having a neoplastic origin include changes in sensation (such as tingling or numbness), normal responses to sensibility tests despite the presence of periapical radiolucency, unsuccessful dental treatments, the presence of diffuse or spreading pain, atypical radiographic lesions (such as poorly defined borders involving multiple teeth or a moth-eaten trabecular pattern), and the absence of identifiable dental etiology (Hargreaves et al).


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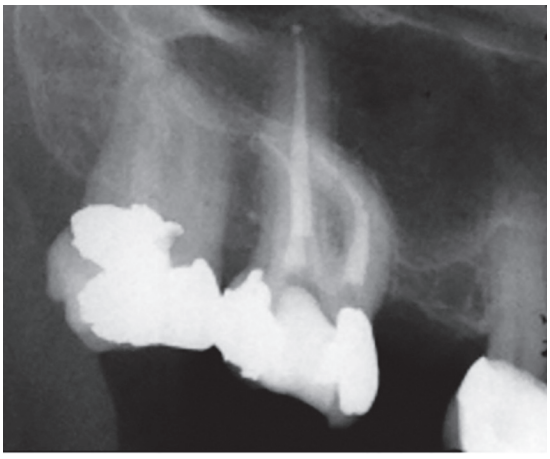
## Example Case Histories

### Case 1

A 54-year-old man presents 3 days after root canal treatment (RCT) on his maxillary right first molar, reporting pain in the maxillary right teeth. He describes the pain as a poorly localized constant dull pain (3/10) with intermittent "throbbing pain" (8/10), increasing in intensity and duration. The pain has been present for over 6 months and is not relieved by his recent RCT. Following RCT, the "throbbing" pain can be slightly ameliorated by 600 mg ibuprofen and two Vicodin (AbbVie) tablets. He has been experiencing increasing nausea and sinus pain over the past 24 hours. He does not feel that his nausea is associated with the Vicodin. He also has become disoriented and has noticed that his right cheek is starting to feel "numb."

Clinical examination reveals slight tenderness to percussion on the maxillary right first molar (RCT 3 days prior), and the other teeth in the sextant respond in short, sharp fashion to cold testing and are not tender to percussion/palpation. Percussion on the maxillary right first molar causes slight tenderness but does not reproduce his chief complaint. There is no evidence of apical bone loss associated with the adjacent teeth. Sensory testing with the sharp and dull end of a cotton tip applicator confirms that the patient is unable to discriminate sharp/dull sensation from the maxillary midline to the right ear.

| Tooth | Maxillary right second molar | Maxillary right first molar | Maxillary right first premolar |
|---|---|---|---|
| Cold | +s | – | +s |
| Percussion | – | Slight | – |
| Palpation | – | – | – |



The periapical radiograph confirms no endodontic etiology, and the lack of endodontic etiology for this patient's progressive symptoms in the maxillary division of the trigeminal nerve should direct the practitioner toward a nonodontogenic etiology. In addition, the

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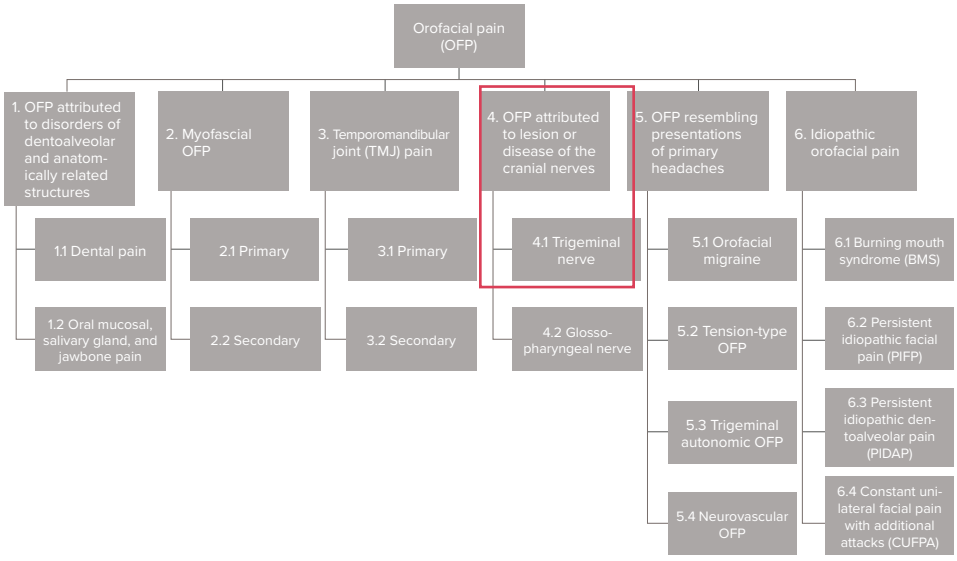
onset of numbness and the lack of symptom resolution following endodontic treatment is suggestive of trigeminal neuropathy.

The use of the ICOP can aid the practitioner in deriving a diagnosis. The first two levels for trigeminal neuropathic pain are highlighted here.


Orofacial pain (OFP)
1. OFP attributed to disorders of dentoalveolar and anatomically related structures
2. Myofascial OFP
3. Temporomandibular joint (TMJ) pain
4. OFP attributed to lesion or disease of the cranial nerves
5. OFP resembling presentations of primary headaches
6. Idiopathic orofacial pain
1.1 Dental pain
1.2 Oral mucosal, salivary gland, and jawbone pain
2.1 Primary
2.2 Secondary
3.1 Primary
3.2 Secondary
4.1 Trigeminal nerve
4.2 Glossopharyngeal nerve
5.1 Orofacial migraine
5.2 Tension-type OFP
5.3 Trigeminal autonomic OFP
5.4 Neurovascular OFP
6.1 Burning mouth syndrome (BMS)
6.2 Persistent idiopathic facial pain (PIFP)
6.3 Persistent idiopathic dentoalveolar pain (PIDAP)
6.4 Constant unilateral facial pain with additional attacks (CUFPA)

The text below (modified from ICOP) describes the criteria for trigeminal neuropathic pain attributed to other disorder.

### 4.1 Pain attributed to lesion or disease of the trigeminal nerve

*4.1.2 Other trigeminal neuropathic pain*

Description: Facial pain in the distribution of one or more branches of the trigeminal nerve caused by another disorder and indicative of neural damage. The primary pain is usually continuous or near-continuous and commonly described as burning, squeezing, aching, or likened to pins and needles. Superimposed brief pain paroxysms may occur but these are not the predominant pain type.

This combination is distinct from that of 4.1.1 Trigeminal neuralgia. There are clinically detectable somatosensory changes within the trigeminal distribution, and mechanical allodynia and cold hyperalgesia/allodynia are common, fulfilling the IASP criteria for neuropathic pain. Allodynic areas may be much larger than the punctate trigger zones present in 4.1.1 Trigeminal neuralgia.

*4.1.2.4 Trigeminal neuropathic pain attributed to other disorder*

Description: Unilateral or bilateral facial or oral pain in the distribution(s) of one or more branches of the trigeminal nerve, caused by a disorder other than those described above, persisting or recurring for more than 3 months and accompanied by other symptoms and/or clinical signs of nerve dysfunction.



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A. Pain, in a neuroanatomically plausible area within the distribution(s) of one or both trigeminal nerve(s), persisting or recurring for > 3 months and fulfilling criteria C and D
B. A disorder other than those identified in 4.1.2.1 to 4.1.2.3, but known to be capable of causing, and explaining, the trigeminal neuropathic pain, has been diagnosed
C. Pain has developed after onset of the presumed causative disorder or has led to its discovery
D. Pain is associated with somatosensory symptoms and/or signs[1] in the same neuroanatomically plausible distribution
E. Not better accounted for by another ICOP or ICHD-3 diagnosis

[1]Somatosensory symptoms or signs may be negative (eg, hypoesthesia and/or hypoalgesia) and/or positive (eg, hyperalgesia and/or allodynia). Comments: Trigeminal neuropathic pain may develop secondary to multiple sclerosis, space-occupying lesion, or systemic disease, with only the clinical characteristics (quality of spontaneous pain, evoked pain, and presence of sensory deficits) distinguishing between 4.1.1.2 Secondary trigeminal neuralgia and 4.1.2 Other trigeminal neuropathic pain.

This case fits the classification:

A. The pain is in a neuroanatomically plausible area within the distribution(s) of one or both trigeminal nerve(s). In this case, the pain is in the maxillary posterior area, in the distribution of the maxillary division of the trigeminal nerve.
B. A disorder other than those identified in 4.1.2.1 (herpes zoster), 4.1.2.2 (postherpetic), or 4.1.2.3 (posttraumatic), but known to be capable of causing, and explaining, the trigeminal neuropathic pain, has been diagnosed. In this case, the patient has no history or signs of a viral infection, and local dental pathosis has been ruled out.
C. Pain has developed after onset of the presumed causative disorder or has led to its discovery. In this case, the array of symptoms (persistent pain following RCT, numbness in the distribution of the maxillary division of the trigeminal nerve, and nausea) are suggestive of a nonodontogenic etiology.
D. Pain is associated with somatosensory symptoms and/or signs in the same neuroanatomically plausible distribution. In this case, there is persistent pain following RCT and numbness in the distribution of the maxillary division of the trigeminal nerve.

Given the likelihood of a nonodontogenic etiology and the rapidly progressing symptoms, the patient was referred for emergency care and was diagnosed with glioblastoma multiforme, which is the most common and most aggressive malignant primary brain tumor, accounting for 52% of all functional tissue brain tumor cases and 20% of all intracranial tumors, with approximately 2 to 3 cases per 100,000. The median survival time for adult patients is approximately 10 months and may be extended to 14 months in patients receiving combined treatment with radiotherapy (Dubrow and Darefsky).

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## Case 2

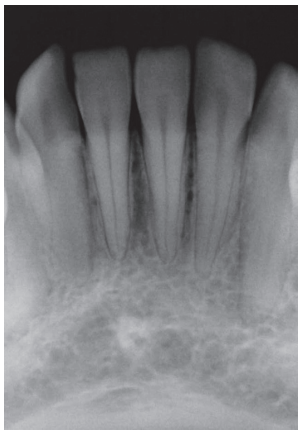
A healthy 32-year-old woman presents with pain in the mandibular anterior teeth that has persisted for over 18 months. She has been attempting to manage the pain with Vicodin. Currently her pain is a constant dull ache in the mandibular incisor area, with average intensity of 6/10, but it can reach 10/10 and have throbbing features. She reports that the pain can spread from the roots of the mandibular incisors "to the top part of teeth" and can evolve to sharp pain.

Her general dentist thought her pain was possibly due to the mandibular right third molar and suggested extraction. However, following extraction of that tooth, the pain in the mandibular incisors persisted. Her primary care physician thought she might have a viral infection and prescribed acyclovir (400 mg, tid) for 10 days, which provided no relief. She had a consult with an ear, nose, and throat specialist, who prescribed a CT scan of the facial region, but nothing abnormal was found. She then sought a consult with a neurologist, who prescribed gabapentin and amitriptyline. The gabapentin helped her tolerate the pain but did not alleviate the pain. The neurologist also prescribed hydrocodone to take at night to alleviate pain and help with sleep. Other alleviating factors include chewing or yelling at sport games. Aggravating factors include stress and exercise.

The dental examination reveals that all mandibular anterior teeth (teeth nos. 22 to 27) respond in short, sharp fashion to cold testing and are not tender to percussion/palpation.

| Tooth | Mandibular left canine (22) | Mandibular left lateral incisor (23) | Mandibular left central incisor (24) | Mandibular right central incisor (25) | Mandibular right lateral incisor (26) | Mandibular right canine (27) |
|---|---|---|---|---|---|---|
| Cold | +s | +s | +s | +s | +s | +s |
| Percussion | – | – | – | – | – | – |
| Palpation | – | – | – | – | – | – |

The periapical radiograph of the mandibular anterior region reveals no evidence of caries, fractures, restorations, or apical bone loss.

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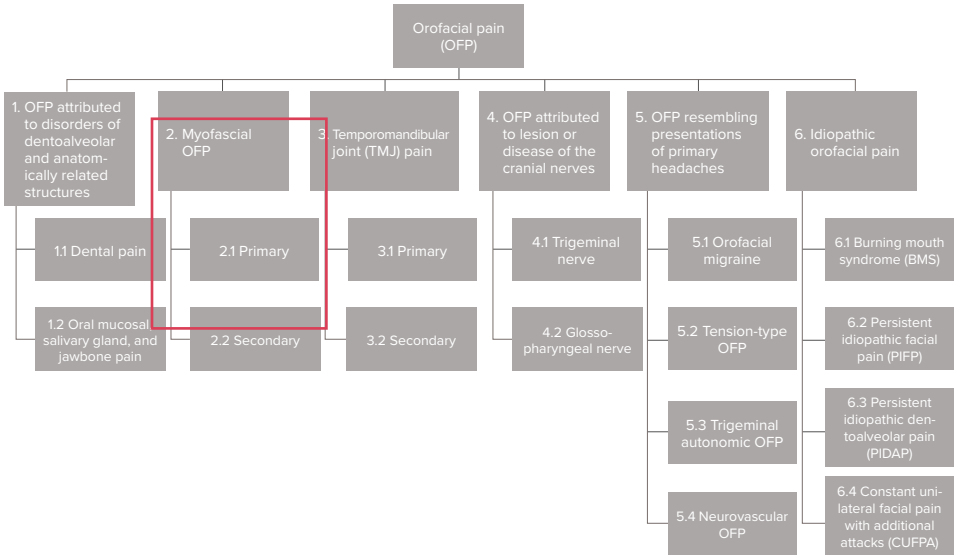
Additional examination reveals severe tenderness to palpation of the anterior belly of the digastric muscles, which reproduces the patient's chief complaint.

Again, the use of the ICOP can aid the practitioner in deriving a diagnosis. The first two levels of primary myofascial orofacial pain are highlighted here.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5.2 Tension-type OFP
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5.4 Neurovascular OFP
6.1 Burning mouth syndrome (BMS)
6.2 Persistent idiopathic facial pain (PIFP)
6.3 Persistent idiopathic dentoalveolar pain (PIDAP)
6.4 Constant unilateral facial pain with additional attacks (CUFPA)

The text below describes the criteria for chronic frequent primary myofascial orofacial pain with pain referral.

### 2.1 Primary myofascial orofacial pain

Description: Pain in masticatory muscles, with or without functional impairment, not attributable to another disorder.

Diagnostic criteria:

A. Myofascial pain fulfilling criteria B–D
B. Occurring in one or more episodes[1] or unremitting
C. Reported in the jaw, temple, ear, and/or in front of the ear, with both of the following:
   1. Confirmation on examination of location(s) in the temporalis and/or masseter muscle(s)
   2. Provoked by either or both of the following:
      a. Palpation of the temporalis and/or masseter muscle(s)
      b. Maximum unassisted or assisted jaw opening movement(s)
D. Modified[2] by jaw movement, function, or parafunction (eg, tooth grinding or clenching)
E. Not better accounted for by another ICOP diagnosis.

[1]Episodes may be single or recurrent within any day, each lasting at least 30 minutes and with a total duration within the day of at least 2 hours.
[2]Pain may be increased or decreased.

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*2.1.2 Chronic primary myofascial orofacial pain*
Description: Mild to moderate levels of deep aching or pressing pain in the masticatory muscles, occurring episodically or unremittingly, often associated with functional impairment such as perceived difficulties in moving the lower jaw, chewing, and/or yawning, etc, and with onset more than 3 months ago. It is often associated with psychosocial distress.
Diagnostic criteria:
A. Myofascial pain fulfilling criteria for 2.1 Primary myofascial orofacial pain and criteria B and C below
B. Onset > 3 months ago
C. Recurring in at least 10 episodes or unremitting.

*2.1.2.2 Chronic frequent primary myofascial orofacial pain*
Diagnostic criteria:
A. Myofascial pain fulfilling criteria for 2.1.2 Chronic primary myofascial pain and criterion B below
B. Occurring on 1–14 days/month on average for > 3 months (> 12 and < 180 days/year)

*2.1.2.2.2 Chronic frequent primary myofascial orofacial pain with pain referral*
Diagnostic criteria:
A. Myofascial pain fulfilling criteria for 2.1.2.2 Chronic frequent primary myofascial pain and criterion B below.
B. Report of pain at a site beyond the boundary of the muscle being palpated

This case fits the classification by fulfilling the following criteria:

A. Myofascial pain confirmed during examination.
B. Patient reports pain is a constant dull ache that has persisted for over 18 months.
C. Pain is reported in the jaw and confirmed on palpation of the anterior belly of the digastric muscle.
D. Pain is modified during exercise.
E. The patient reports pain in the mandibular anterior teeth.

The patient was referred to an orofacial pain specialist for management of chronic primary myofascial orofacial pain.

These cases demonstrate the importance of proper diagnosis. In the first case, continued endodontic treatment would have prolonged the patient's suffering and delayed the diagnosis of a potentially life-threatening condition (glioblastoma multiforme). In the second case, continued endodontic treatment would have prolonged the patient's suffering and potentially exacerbated her chronic myofascial pain.


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## Conclusion

When encountering a patient with symptoms of tooth pain, clinicians should challenge themselves to look at the whole person instead of immediately zooming in on the tooth. Despite a common perception, the key to a correct diagnosis of nonodontogenic toothache is not in the clinical examination but in a very attentive, structured, and detailed history of the presenting pain and its contributing factors. The thorough history will help the provider associate their patient's pain with one of the four (musculoskeletal, neurovascular, neuropathic, or nociplastic) major categories of nonodontogenic pain conditions. The aim of the clinical examination is then to verify the initial differential diagnostic hypothesis and to reproduce the patient's chief complaint. Even if obvious pulpal-periapical pathosis is evident, a significant proportion of patients experience mixed pain; if a nonodontogenic component of pain is overlooked, the patient may interpret the residual pain as persistent "tooth" pain after an otherwise successful endodontic treatment. By far the most common cause of nonodontogenic toothache is TMD pain, and therefore dentists need to be familiar with and utilize validated diagnostic criteria for TMDs (ie, DC/TMD) in their practice. Finally, there is a need for the collaboration of endodontists and general dentists with orofacial pain specialists to appropriately manage patients with nonodontogenic pain.

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# Appendix 1

## SELF-CARE REGIMEN TO EASE TMDs

*Temporomandibular disorder (TMD)* is an umbrella term that describes several different conditions that affect the jaw and face muscles, the jaw joints (temporomandibular joints [TMJs]), or both. Common symptoms include muscle pains, jaw joint pains, noises, movement irregularities, and headaches. Some pains can be caused by one of these body parts but felt in a different location, such as a tooth. This is called *referred pain*.

Your dentist has evaluated you and believes at least a part of your tooth pain is coming from one of these other body parts associated with TMDs. For the tooth pain to improve, we need to focus on treating the body part causing the pain. The following recommendations have been shown to significantly ease pains associated with TMDs when consistently and correctly performed as prescribed.

- **Avoid touching your teeth together:** If you find yourself resting with your teeth even lightly touching together, this is considered clenching and puts strain on the jaw joints and muscles. Practice resting with the tip of your tongue lightly resting against the roof of your mouth, your teeth slightly separated, and your jaw/face relaxed. If you say the letter "n" the tongue often goes to a comfortable position. Set reminders for yourself to closely monitor your jaw position during your waking hours so that you maintain your jaw in a relaxed, comfortable "n" position throughout the day.
- **Eat a pain-free diet:** Avoid chewy and hard foods like baguettes, jerky, steak, hard or sticky candies, etc. Do not overextend your jaw by taking large bites. All food should be cut into pieces no bigger than the tip of your thumb. Chew your food equally on both sides of your mouth using your back teeth. This takes practice.
- **Avoid activities that put additional stress on your jaw:** This includes chewing gum, cheek biting, chewing on objects (pens/pencils), resting with your face on your hand, tooth grinding, biting cheeks/lips/fingernails, pushing your tongue against teeth, or checking your bite. Ask friends/family to see if they notice you doing any unusual habits with your jaw or if they see you clenching or grinding your teeth.
- **Avoid stomach sleeping:** This places your head and neck muscles in a strained position while you sleep. Side sleeping can be okay, but make sure your neck is supported and that you are not resting with too much pressure on your lower jaw.
- **Avoid caffeine intake:** Caffeine is a drug that causes muscles to tense. If you cannot avoid it altogether, limit your intake to less than two cups per day.
- **Apply moist heat:** Applying moist heat to the jaw muscles along the sides of the head and along the cheeks for 15 to 20 minutes two to four times per day will help to alleviate pain. Also try using ice wrapped in a very thin cloth for 5 to 10 minutes. Heat or ice can reduce joint or muscle pains and relax the muscles.
- **Perform any recommended exercises as prescribed by your provider.**

Most people experience gradual symptom improvement over several weeks. If your pain worsens, contact your dentist to determine the next steps.

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# Appendix 2

## EXERCISES TO EASE TMDs

Each recommended exercise should be done three to four times per day. Be sure to spread sessions out over the day. You may also use these any time you notice yourself clenching or tensing your jaw. It is often helpful to apply heat or cold packs (your preference) to the areas for about 10 to 15 minutes immediately before and/or after the exercises.

Breathing throughout these exercises should be slow and controlled. We suggest practicing square breathing as a starting point, where you slowly inhale over 4 seconds, pause your breath for 4 seconds, exhale over 4 seconds, pause your breath for 4 seconds, inhale 4 seconds, etc. After you try this, find what variation works for you and is comfortable and relaxed. Some people prefer to initially practice just the breathing portion alone.

If exercises are causing mild pain, first ease off on the pressure or number of repetitions performed. If pain persists or is severe, discontinue and contact the office.

### Rotation

1. Gently put the tip of your tongue on the roof of your mouth near the back of your throat (to help keep your jaw from jutting forward). Keep it there for the entire exercise.
2. Check the position of your dental midlines (how the front two teeth on the top and front two teeth on the bottom line up when the teeth meet). Keep your natural alignment throughout the exercise. (Don't let your jaw wander side to side. Using a mirror helps.)
3. Monitor the position of your TMJs with your index fingers over the joints throughout the exercise. You should feel minimal movement. If you feel the jaw "jump" or slide or a deeper indentation forming by your ear, you are opening too wide.
4. Slowly and with control, open and close your mouth 10 times, then rest. Repeat this three times.

This exercise should require you to open no bigger than two fingertip widths. Chewing within this shortened range is often helpful to reduce jaw pain with eating.

### Relaxation

1. Locate the tight muscles. You may briefly and lightly touch your teeth together while touching the temple area of your head and then the cheeks with your fingers to locate the muscles that are sore. The muscles will flex as your teeth touch. These are the areas you want to focus on relaxing. (Only do this step if you are unsure of where to massage. Once you know the areas, this step is not necessary.)
2. Let your jaw relax (ie, drop/sag jaw). Do not try to actively open your mouth; just let your jaw relax. Your lips may even part a little.
3. Take a slow, deep breath in as you push the palms of your hands gently, but firmly, against the sides of your head, starting slightly above where you felt the bulk of the muscles. The force you use should NOT be painful. Slowly exhale over a minimum of 3 to 4 seconds as you push in and gently pull your hands down, allowing your hands to slide over the muscles.
4. When your hands reach the corners of your eyes, take another slow, deep breath in and slowly exhale over another 3 to 4 seconds while sliding your hands down the sides of your face to below your jaw. The jaw should be sagging open by the end. Do this as many times as necessary until the jaw and face feel relaxed.

This is a great exercise to do a few times whenever you feel your jaw tense.

### Passive finger stretch

1. Place one fingertip sideways between your front teeth. Do not bite your finger; it is only there as a spacer. Keep your whole focus on relaxing your jaw while you slowly and deeply breathe in and out, using your free hand to massage any tight spots. Every time you slowly breathe out, try to relax your jaw a little more. Do this for 30 to 60 seconds.
2. Rest 30 to 60 seconds, then repeat with one knuckle as a spacer. Rest, and then repeat with two fingertips as a spacer. Rest, then repeat with two knuckles as a spacer. This should be a stretch, but not painful. If any level is painful, practice to the level below that and gradually work your way up to two knuckles. Do not go beyond two knuckles; this may overstretch your jaw, leading to other complications.

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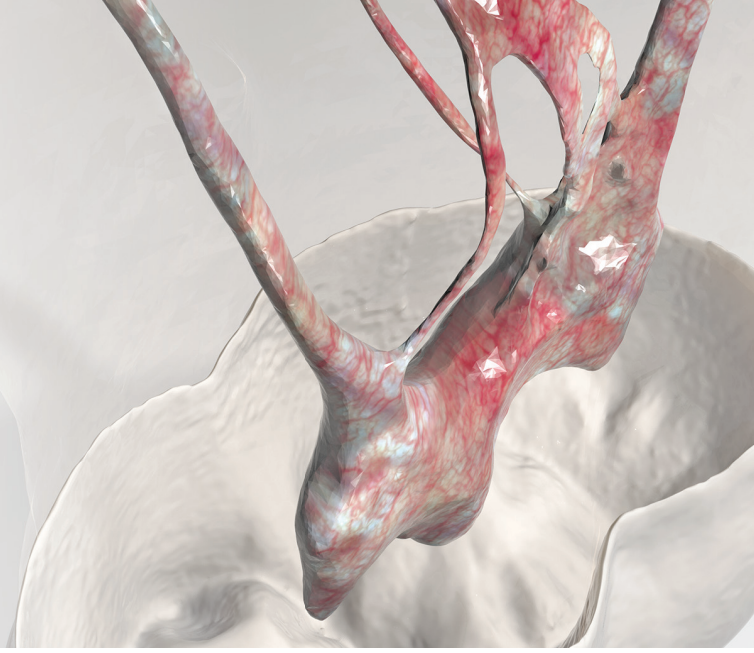
# 16

# INTERRELATIONSHIP OF THE PULP AND PERIAPICAL TISSUES WITH SYSTEMIC DISEASE

Anita Aminoshariae
James L. Gutmann



Endodontic practice encompasses the management of inflammatory processes that can be triggered by microbial insult to the pulp and the periapical tissues (Hahn and Best, Siqueira et al 2009). Apical periodontitis is a complex inflammatory response to a slow and constant microbial insult (Hahn and Best, Hahn and Liewehr 2007a, Hahn and Liewehr 2007b). The chronic inflammatory reaction to this microbial insult involves several inflammatory mediators, morphologic changes, neurogenic inflammation, and immunologic activation. Microbial insult activates innate immunity, and if the innate immunity is unable to eradicate the insult, then acquired immunity is elicited (Hahn and Liewehr 2007a). As a result of this chronic and constant insult to microbial challenge, degradation of pulpal and periapical tissue occurs, resulting in a nonspecific immune response (Marending et al) that is associated with a number of systemic diseases (Khalighinejad et al 2016).

The long-term effect of periapical pathosis on systemic disease remains elusive (Khalighinejad et al 2016). However, the effect of apical inflammation on systemic immune response cannot be underestimated. More importantly, the individual's susceptibility to microbial and inflammatory diseases based on their genetics must always be considered (Aminoshariae and Kulild). This chapter reviews the most current understanding of the relationship between the pulp and systemic disease.

## Pathophysiology of Pulpal Disease

The pathophysiology of pulpal and periapical disease has been thoroughly described in the previous chapters. Briefly, the origin of pulpal disease is microbial invasion, primarily by gram-negative anaerobic species (Siqueira et al 2004), as discussed in chapter 6. The microbial invasion elicits a complex immunologic response in the pulp. Subsequently, if this insult is unresolved, the apical tissue is viewed as the secondary defense mechanism against caries, trauma, and microbial invasion (Orstavik et al) (see chapter 8).

This inflammatory reaction is unique to the host's genetic makeup. The association between apical periodontitis and genetic polymorphism suggests that biologic modifiers in certain individuals may challenge the immunoinflammatory response (Aminoshariae and Kulild). In short, agents of the innate immune response such as dendritic cells, macrophages, and neutrophils start the proinflammatory process by activating nuclear factor κB (NFκB) in a proinflammatory signaling pathway that stimulates cytokines and chemokines. This in turn triggers interferon-γ (IFN-γ), interleukin 17 (IL-17), IL-1, and tumor necrosis factor (TNF). Concurrently, the cells in the periapical tissue and alveolar bone are stimulated to release proinflammatory cytokines, chemokines, and inflammatory mediators.

The unresolved microbial insult with proinflammatory response elicits acquired immunity by activating cell-mediated and humoral response. Once the neighboring cells in the periapical tissue and alveolar bone are stimulated by innate and acquired responses, receptor activator of NFκB ligand (RANKL) initiates bone resorption (Bostanci et al).

Compared to a healthy individual, a patient with apical periodontitis has higher values of circulating white blood cells, C-reactive protein (CRP), and immunoglobulins (Gomes et al 2013). As such, local inflammation may modify and overburden the systemic inflammatory response. It is important to note that to date no causation

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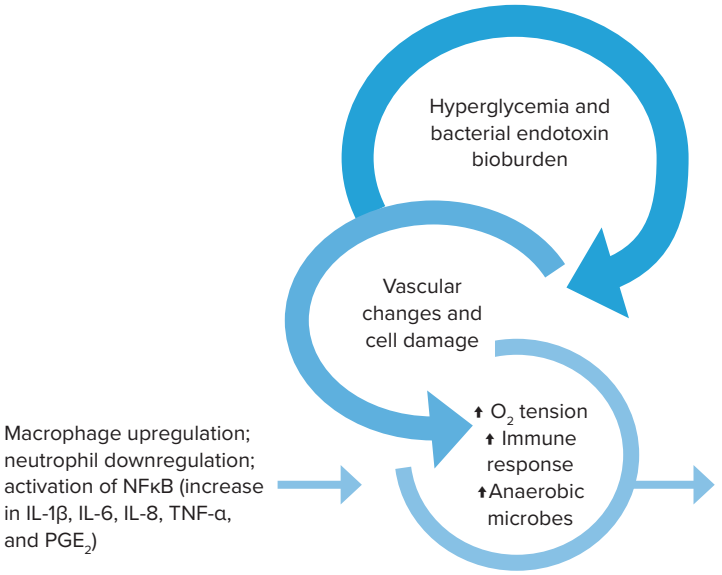
Hyperglycemia and bacterial endotoxin bioburden
Vascular changes and cell damage
↑ $O_2$ tension
↑ Immune response
↑ Anaerobic microbes
Macrophage upregulation; neutrophil downregulation; activation of NFκB (increase in IL-1β, IL-6, IL-8, TNF-α, and $PGE_2$)
Endotoxemia; beta cell apoptosis; insulin resistance

◀ **FIG 16-1** Biologic association between DM and periapical lesions.

has been established. Additionally, in a systematic review evaluating the long-term use of medications and prevalence of endodontic disease, the authors reported that despite the low level of evidence, some medications like infliximab, adalimumab (for at least 6 months), metformin, and statins had a positive reinforcement on apical healing (Alghofaily and Fouad).

The concept of low-grade constant periapical infection has been linked to systemic comorbidities such as diabetes and cardiovascular disease (Khalighinejad et al 2016). Although there is no strong evidence to support that pulpal infection may impact the systemic health of a healthy and not otherwise medically compromised individual, there is also no evidence to refute this (Siqueira et al 2011a, Aminoshariae et al 2018).

## Association of Pulpal and Periapical Disease with Systemic Disease

### Diabetes mellitus

Diabetes mellitus (DM) is a metabolic disorder that has macro- and microvasculature components. The disease is characterized by insulin deficiency or resistance. Type 1 diabetes is characterized by cell-mediated autoimmune response and the destruction of pancreatic beta cells that results in insulin loss. Type 2 diabetes is characterized by insulin resistance and hyperglycemia. There is evidence that the inflammatory response damages the normal structure of beta cells, inducing pancreatic apoptosis (Montane et al).

These changes induce alteration of immunologic cells, upregulation of macrophages and polymorphonuclear leukocytes, and downregulation of growth factors. Specifically, the accumulation of advanced glycation end products triggers a proinflammatory response that initiates and releases cytokines, chemokines, vascular cell adhesion molecule-1, and inflammatory mediators into the bloodstream. Subsequently, these factors alter endothelial function, resulting in impaired vasculature (Segura-Egea et al 2016, Segura-Egea et al 2015) (Fig 16-1).

The association between the pulp and DM has been established in a number of cross-sectional studies (Gonzalez Marrero et al, Bender and Bender), and the association between apical disease and DM has also been well documented (Segura-Egea et al 2016, Fouad 2003, Yip et al). Again, causation has yet to be proven. Furthermore, uncontrolled DM, poor circulation, impaired immunoinflammatory response, and microbial challenges contribute to poor wound healing, persistent infection, and nonhealing endodontic outcomes (Segura-Egea et al 2015, Aminoshariae et al 2017, Arya et al, Nagendrababu et al, Jakovljevic and Duncan, Segura-Egea et al 2023). The impact of any endodontic intervention (nonsurgical/retreatment/surgical intervention) on DM has yet to be fully studied. In an animal study, apical periodontitis resulted in an increased level of HbA1c (Cintra et al). A double-blinded clinical trial evaluated the effect of endodontic treatment on systemic inflammation in patients with type 2 diabetes (Wang et al). The authors reported that endodontic treatment contributed to apical healing, lowering the number of inflammatory cytokines and improving glycemic control, but a continued reduction in hemoglobin glycation (HbA1c) and inflammatory factors at 6 months was not observed. The question of whether nonsurgical endodontic treatment (or any


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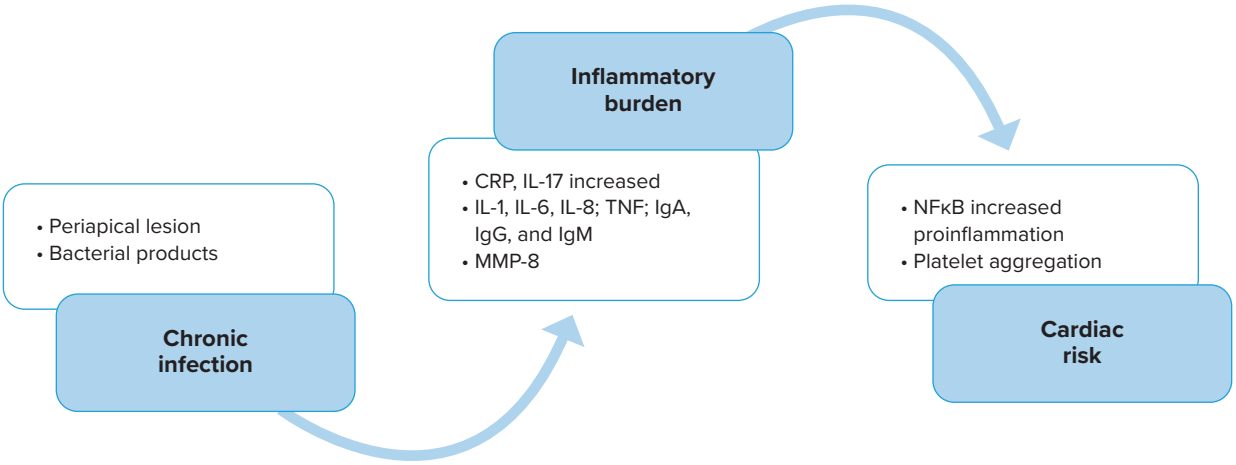
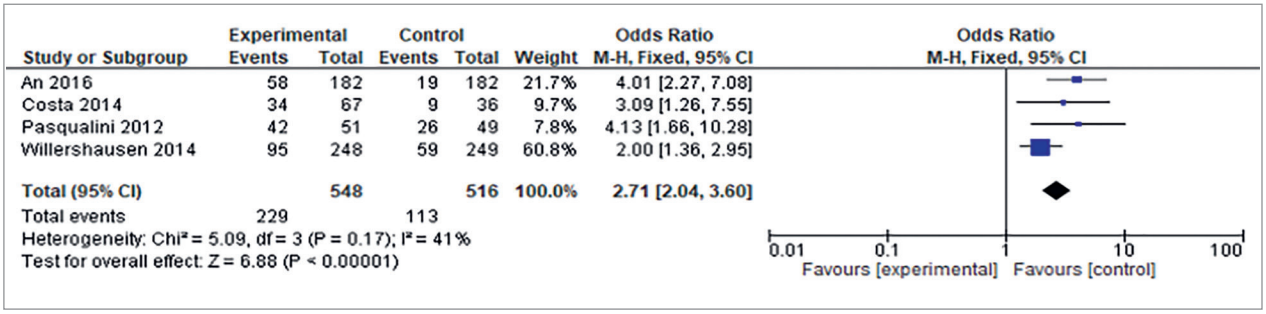



▲ **FIG 16-2** Biologic association between CVD and periapical pathosis (Gomes et al 2013, Garg and Chaman, Georgiou et al). MMP-8 = matrix metalloproteinase 8; Ig = immunoglobulin.


Experimental
Control
Odds Ratio
Odds Ratio
Study or Subgroup Events Total Events Total Weight M-H, Fixed, 95% CI
M-H, Fixed, 95% CI
An 2016 58 182 19 182 21.7% 4.01 [2.27, 7.08]
Costa 2014 34 67 9 36 9.7% 3.09 [1.26, 7.55]
Pasqualini 2012 42 51 26 49 7.8% 4.13 [1.66, 10.28]
Willershausen 2014 95 248 59 249 60.8% 2.00 [1.36, 2.95]
Total (95% CI) 548 516 100.0% 2.71 [2.04, 3.60]
Total events 229 113
Heterogeneity: Chi² = 5.09, df = 3 (P = 0.17); I² = 41%
Test for overall effect: Z = 6.88 (P < 0.00001)
0.01
0.1
1
10
100
Favours [experimental]
Favours [control]

▲ **FIG 16-3** Association between CVD and periapical lesions according to case-control studies (odds ratio: 2.71; CI [2.04–3.60], $P < .001$).

endodontic intervention or pulp treatment) would lower HbA1c levels needs to be fully explored and definitively answered in long-term outcome studies.

## Cardiovascular disease

The association between apical periodontitis and cardiovascular disease (CVD) has been discussed in several cross-sectional case-control studies and systematic reviews (Aloutaibi et al, Khalighinejad et al 2016, Koletsi et al, Cotti et al 2011a, Cotti et al 2011b, Noites et al). Although there may be a substantial association linking apical periodontitis to CVD, there is no evidence proving causation.

The pathophysiology of this association is probably best described by microbial invasion and stimulation of innate and adaptive cells leading to an increase in bioburden and activation of the proinflammatory response, which then triggers the NFκB pathway (Garg and Chaman). This leads to further inflammatory response, including the release of inflammatory mediators and CRP as well as platelet aggregation (Georgiou et al). Enhanced activity of NFκB has been linked to the initiation and progression of atherosclerotic plaque (De Martin et al) (Fig 16-2).

The association of endodontic pathosis (more specifically pulpal and periapical pathosis) and CVD has been reported in many case-control and cross-sectional studies (Cotti et al 2011a, Pasqualini et al, Willershausen et al, Liljestrand et al 2016, An et al, Virtanen et al) (Fig 16-3). One cross-sectional study explored the combination of endodontic and periodontal disease parameters as main exposures in stroke patients; the authors reported that the combination of oral inflammatory burden (endodontic pathosis and alveolar bone loss) strengthened the prevalence of carotid atherosclerosis (Leão et al).

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The question of whether CVD impacts treatment outcomes has been the subject of much interest recently (Laukkanen et al, Ng et al, Aminoshariae et al 2017, Aminoshariae et al 2020a). A systematic review of longitudinal studies reported that CVD negatively affected the outcome of endodontic treatment by 67% (Aminoshariae et al 2020a). The original studies, however, did not control for several confounding variables, including periodontal disease. Controlling for periodontal disease is an important parameter; in fact, a consensus report from the World Heart Federation stated significant associations between periodontitis and CVD (Sanz et al).

The reverse effect of endodontic pathosis on CVD has also been studied (Gomes et al 2013, Aminoshariae et al 2018, Caplan et al 2006, Caplan et al 2009). The current literature suggests a lack of scientific evidence to support or reject an effect or impact of endodontic pathosis (pulpal or periapical pathosis) on CVD (Aminoshariae et al 2020a). In a microbiologic study of nonsurgical endodontic treatment, the authors took the blood samples of 21 patients with periapical lesions at high risk for infective endocarditis and 11 patients with no risk for infective endocarditis (Reis et al). The patients at risk for infective endocarditis received prophylactic antibiotic therapy. The authors reported no detectable bacteremia by cultural DNA after the treatment.

Whether any type of endodontic treatment would decrease the incidence of CVD has been studied in an epidemiologic study, and the authors reported no significant association (Cowan et al). In this self-report of endodontic therapy, the recall of the participants was the only source of information for the patient's outcome. Because there is recall bias in this model, well-conducted studies are needed to study whether endodontic treatment has an impact on CVD.

Nonetheless, in a case-control study, periapical pathosis was associated with a first myocardial infarction (MI) in patients aged 28 to 64 years, and endodontic treatment was associated with a first MI in patients aged 65 to 75 years (Sebring et al), suggesting an association. In another longitudinal cohort study with a mean of 17.4 years of follow-up, the authors evaluated whether a periapical lesion or endodontic treatment would impact the incidence of CVD; they reported that endodontic pathosis and endodontic treatment were a predictor of CVD, independent of periodontal disease (Gomes et al 2016).

Interestingly, missing teeth have been associated with CVD in several studies (Cowan et al, Sebring et al, Peng et al, Liljestrand et al 2015). From the standpoint of saving teeth, it seems logical that endodontic treatment would benefit patient health and mortality, but more well-conducted studies should be performed to ascertain the cost and benefit of endodontic treatment and CVD.

## Chronic kidney disease

In recent years, many studies have explored the association between pulp stones and systemic conditions, with inconsistent findings (Patil and Sinha, Patil, Gabardo et al, Tarim Ertas et al, Galili et al). Some authors have reported that there may be an association between pulp stones and kidney stones, and others have not reported any association (Tarim Ertas et al). However, these studies all had small sample sizes with many confounding variables that were not controlled or adjusted for, such as oral hygiene status, ferrule effect, number of restorations, trauma, age, dental status, and periodontal health. Thus, well-conducted studies are required to confirm this association.

There is a scarcity of studies exploring the association between chronic kidney disease (CKD) and endodontic disease (Khalighinejad et al 2017b, Patil and Sinha). With CKD, the calcium-phosphate complex is out of balance. This is due to poor glomerular function and lack of 1,25-dihydroxycholecalciferol synthesis, which leads to demineralization. The decreased glomerular function results in high serum phosphate, low serum calcium, and poor absorption of vitamin D, which causes increased production of parathyroid hormone (PTH). Secondary PTH activates TNF and IL-1, which trigger bone remodeling and increased serum calcium as the calcium is leached out of cortical bone into the bloodstream.

Whether CKD is associated with nonhealing apical periodontitis remains to be studied. Furthermore, the impact of treatment procedures on CKD needs to be evaluated.

## Viruses

Many authors have explored the association between viruses and the pulp and periapical lesions (Sabeti et al 2009, Sabeti et al 2012, Sabeti et al 2003a, Sabeti et al 2003b, Slots et al, Yazdi et al, Zargar et al). Herpes simplex virus (Zargar et al), human cytomegalovirus (Slots et al, Sabeti et al 2009, Sabeti et al 2012, Yazdi et al), Epstein-Barr virus (Slots et al, Sabeti et al 2012), and HIV (Glick et al 1991, Glick et al 1989, Elkins et al) have all been detected in the pulp and periapical tissue.

In a randomized double-blind study, the authors investigated the analgesic effect of 500 mg of valacyclovir twice a day for 3 days for acute apical abscesses (Sabeti et al 2021). Twenty patients were stratified into two groups: *(1)* antibiotics and antiviral medication or *(2)* antibiotics and placebo. No other endodontic treatment (such as incision and drainage) was performed. The authors reported that antiviral medication in conjunction with antibiotics was a promising adjunctive agent in emergency patients with acute apical abscess. The findings show that viruses are an integral part of the microbial profile of the pulp and periapical abscesses.

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The outcomes of nonsurgical root canal treatment in patients with HIV have also been studied, and the authors reported that HIV infection does not result in nonhealing endodontic treatment (Shetty et al, Suchina et al, Aminoshariae et al 2017, Alley et al). Well-conducted studies are needed to explore whether nonsurgical root canal treatment would have any impact on patients with viral diseases.

## Pregnancy

Many authors have reported an association between pulpal/periapical inflammation and adverse pregnancy outcomes (Jakovljevic et al 2021b, Khalighinejad et al 2016, Khalighinejad et al 2017a). While the reason for this association could be multifactorial, the microbial infection and subsequent inflammatory burden from pulpal and periapical pathosis have been linked to adverse pregnancy outcomes such as preeclampsia and low birth weight. A systematic review of two case-control studies and one cross-sectional study reported with fair quality of evidence that maternal apical periodontitis is associated with adverse pregnancy outcomes (Jakovljevic et al 2021b).

## Gastroenterology

Several case-control studies have explored the association between inflammatory bowel disease (IBD) and periapical lesions (Piras et al, Poyato-Borrego et al, Segura-Sampedro et al). The authors reported that subjects with IBD have significantly higher prevalence of periapical lesions compared with healthy controls. Another study examined the prevalence of periapical lesions in patients with cirrhosis (Grønkjær et al); the authors reported that the presence of a periapical lesion was associated with a higher prevalence of cirrhosis-related complications. The reason for these findings is speculative and perhaps multifactorial, potentially including pathogenesis of the proinflammatory cells, poor diet, alcohol usage, and lack of proper oral health and care.

Osteoprotegerin concentration and alterations in RANKL could also explain the association between periapical lesions and gastroenterologic problems (Moschen et al). Osteoprotegerin affects bone turnover and differentiation, and its concentration has been correlated with prognosis in IBD and gastrointestinal carcinoma (De Voogd et al).

In a longitudinal cohort study (6-month follow-up), the authors explored the radiographic healing of patients with IBD taking biologic medications compared to healthy controls (Cotti et al 2018). The authors reported that radiographic healing was comparable between the groups. Well-conducted studies with long follow-ups are required to substantiate the association, prognosis, and healing of endodontic treatment in these patients.

## Blood disorders

### Sickle cell anemia

Sickle cell anemia (SCA) is characterized by a congenital abnormality of red blood cells that results in poor blood oxygenation. A case-control study reported on the association between SCA and pulp necrosis (Costa et al 2013), and a case series investigation reported that patients with SCA have a significantly higher incidence of pulp necrosis in the absence of microbial infection (Costa et al 2021).

The pathophysiology of this phenomenon has been associated with the proinflammatory state (Ferreira et al). The proinflammatory response manifests itself with severe chronic hemolysis and vaso-occlusive complications combined with vasculopathy and consequent pulp necrosis (Costa et al 2020). The authors reported a strong association between pulp necrosis and increased death rates in their study (Costa et al 2020).

### Inherited coagulation disorder

A case-control study of 58 patients with hemophilia A, hemophilia B, or von Willebrand's disease and 58 healthy controls with no disease reported on the prevalence of apical periodontitis and inherited coagulation disorder. After multivariate logistic regression analysis, the authors reported that patients with inherited coagulation disorders had higher odds for periapical radiolucency (Castellanos-Cosano et al).

## Smoking

Several studies have linked pulpal and periapical pathosis with smoking (Aminoshariae et al 2020b, Cabanillas-Balsera et al). This association is likely due to changes in the microbial flora and microbiome, increased caries risk, poor blood circulation and bone turnover, activation of RANKL, and bone destruction (Aminoshariae et al 2020b). The current best evidence supports that smoking adversely affects pulp circulation and results in necrosis (ie, a high prevalence of periapical lesions) (Aminoshariae et al 2020b).

## Polymorphism and endodontic disease

Genetic variation dictates different responses in immunology, inflammation, initiation and progression of the disease, and healing capacity (Jakovljevic et al 2021a, Morsani et al). Several studies have investigated single-nucleotide polymorphisms in proinflammatory cytokines, chemokines, matrix metalloproteinases (MMPs), and bone resorption regulators as genetic modifying biomarkers (Amaya et al, Dill et al, Evrosimovska et al, Morsani et al, de Sá et al).

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An umbrella review was performed to ascertain the quality and certainty of evidence supporting the association between polymorphism and apical periodontitis. The authors reported that with limited evidence and high heterogeneity among the studies, the carriers of specific genotypes and alleles of TNF-α -308 G > A and IL-1β + 3954 C/T gene polymorphisms might be susceptible to the initiation, progression, and persistence of apical lesions (Jakovljevic et al 2022).

Recent studies have reported that individual genetic variation contributes to the initiation and progression of disease, with different outcomes (Aminoshariae and Kulild, Colavite et al 2019, Fouad et al, Jakovljevic et al 2021a, Rôças et al, Siqueira et al 2011b). This has introduced the concept of genome-wide association studies (GWAS) in terms of genetic risk factors (Fouad 2017). In other words, the researchers were searching the entire genome looking for a specific gene that may be involved in disease risk. The concept of GWAS could transform all phases of endodontics to advanced personalized medicine with individual risk prediction, optimizing therapy and identifying therapeutic drugs and treatment. Infected and inflamed pulps might express different gene profiles that could significantly affect the immune response complex. The concept of personalized medicine might enhance our understanding of pulpal infection and disease, allowing us to bridge this biologic response with targeted treatment (Galicia et al).

## Conclusion

It is important to note that despite the strength of association between apical periodontitis and systemic disease, no causation has been reported or can be confirmed. Confounding variables remain the most challenging issue to address in interpreting the data. Most of the available studies had high heterogeneity and low sample size and included periodontal disease, ferrule effect, body mass index, smoking, alcohol impact, genetic variability, epigenetics, and many other covariants that may have impacted the results. As such, the results of these observational studies should be regarded with caution. Systematic reviews cannot correct for the inherent weaknesses in the original studies.

The effect of the microbiome on the genetic manifestation of disease should be better explored to help understand the relationship between apical periodontitis and systemic disease. Also, the role of GWAS should be better evaluated in the future.

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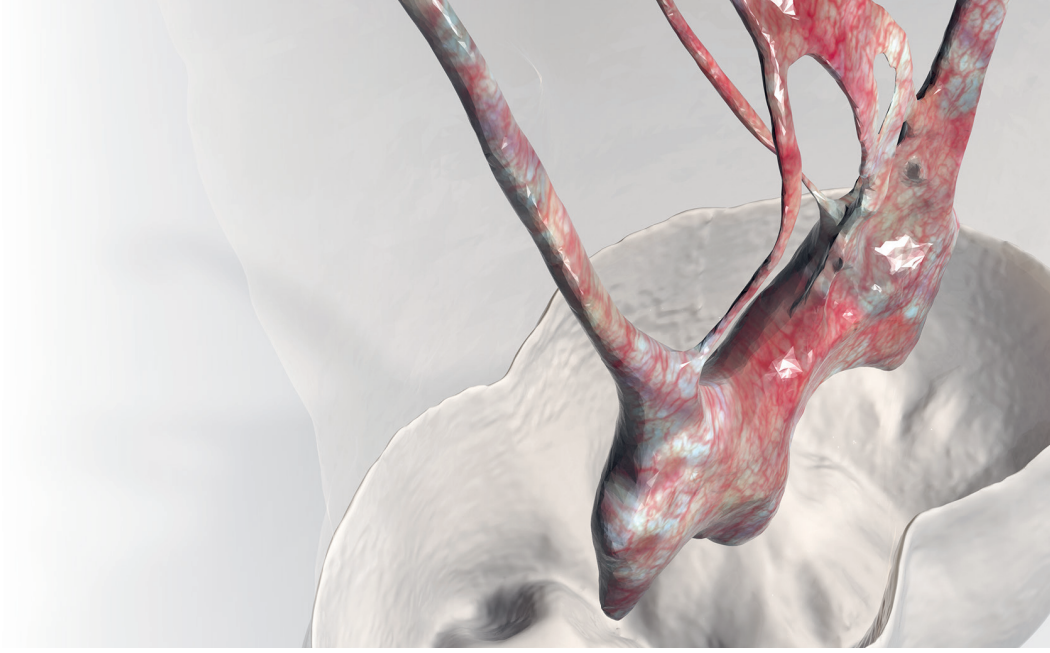
# 17

# ROOT CANAL MORPHOLOGY

Ronald Ordinola Zapata
Jorge N. R. Martins
Marco A. Versiani



Root canal morphology is one of the foundations of the art and science of endodontic treatment. Ethnicity, age, pulpal irritants, resorption, and calcifications can have a profound effect on the anatomical configurations of the human dentition, which exhibits a broad range of morphologic forms. As such, it is necessary to have a comprehensive understanding of the pulp chamber and canal morphology, as well as their variations. This reduces the likelihood of missing anatomy and iatrogenic mishaps, hence improving the outcome of endodontic treatment.

In the past two decades, technical advances have enabled the use of 3D imaging in the study of root canal anatomy. CBCT and high-resolution microcomputed tomography (microCT) are some examples. These technologies provide a nondestructive and repeatable process to produce quantitative and qualitative morphometric data of the root canal system. This chapter describes the principal findings of laboratory and CBCT investigations on the anatomy of the root and root canal system utilizing contemporary imaging techniques.

## Root Canal Morphology

A fundamental principle in pulp-root anatomy is that the form of the pulp canal system mirrors the crown and root surface outlines. The root canal system can be divided into the *pulp chamber*, defined as the portion of the pulp space within the anatomical crown of the tooth, and a root canal space (Versiani et al 2018a). According to the American Association of Endodontists (AAE), the *root canal* is defined as a passage or channel in the root of the tooth extending from the pulp chamber to the apical foramen (American Association of Endodontists [AAE]). The root canal may be large, narrow, or calcified; it may include lateral branches, multiplanar curvatures, interconnections, and apical ramifications; and its overall morphology is irregular.

### Pulp chamber

The pulp chamber is a cavity situated in the center of the crown and usually resembles the shape of the crown surface. Due to the absence of a pulpal floor in anterior teeth, the pulp chamber and root canal are continuous. The pulp chamber of multirooted teeth has a square form with six sides: the floor, the roof, and four axial walls (mesial, distal, buccal, and lingual). The floor separates the pulp chamber space from the root canals. The pulp chamber roof usually presents projections or prominences associated with the cusps, mamelons, or incisal ridges, known as *pulp horns*. These projections are more evident in children and young adults (Versiani et al 2018a). As described in earlier chapters, continuous dentin formation by primary (physiologic or reactive) odontoblasts causes a reduction in pulpal canal space diameter. Without magnification or 3D imaging, the pulp chamber and root canal orifices may not be readily apparent, which may provide further difficulties for the clinician (Tjäderhane). Deutsch and Musikant completed morphologic measurements in maxillary and mandibular molars. They found that, on average, the distance from the cusp tip to the pulp chamber roof is 6 mm, while the distance from the pulpal floor to the roof is approximately 3 mm. The pulp chamber is found at the level of the cementoenamel junction (CEJ) in 97% to 98% of molars.

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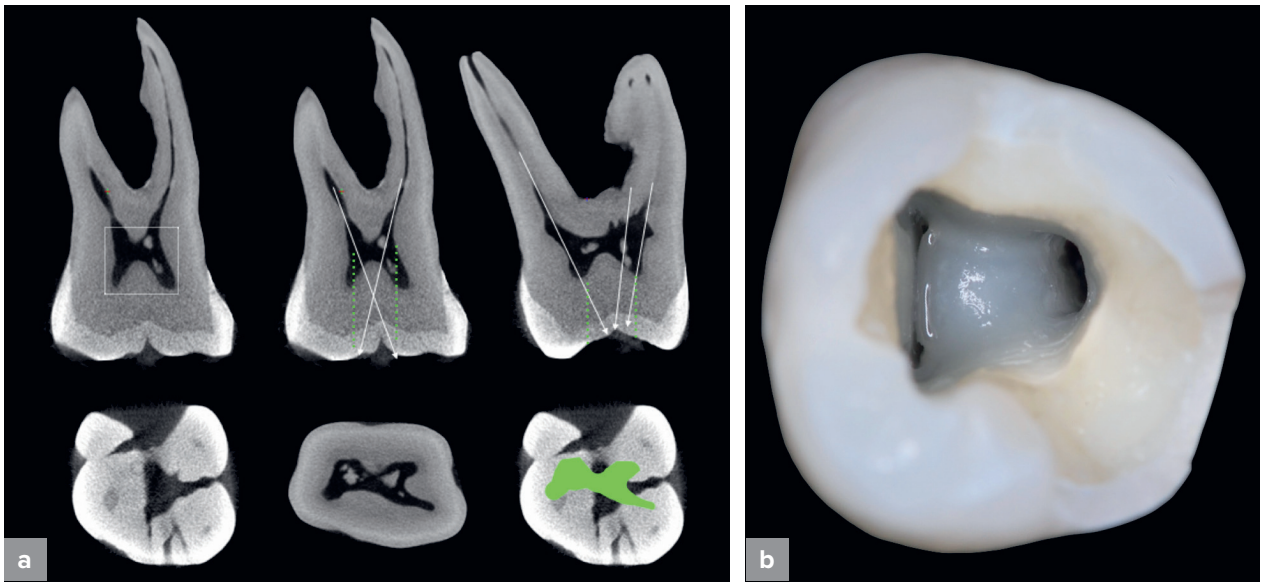


▲ **FIG 17-1** *(a)* Laws of centrality, concentricity, and orifice locations observed through microCT sagittal and axial sections in a maxillary first molar. The projections of the root canal axes *(white lines)* converge into the center of the anatomical crown *(green area)*. *(b)* The law of color change can be observed in the pulp chamber of an extracted mandibular molar. The floor is darker than the root canal walls, and the orifices are always located at the junction between the floor and the walls. (Modified from Ordinola-Zapata et al 2018.)

Krasner and Rankow identified traits shared by anterior and posterior teeth that might assist clinicians in locating the pulp chamber and root canal orifices (Fig 17-1):

- **Law of centrality:** The floor of the pulp chamber is always located in the center of the tooth at the level of the CEJ.
- **Law of concentricity:** The walls of the pulp chamber are always concentric to the external surface of the tooth at the level of the CEJ (ie, the external root surface anatomy reflects the internal pulp chamber anatomy).
- **Law of the CEJ:** The distance from the external surface of the clinical crown to the wall of the pulp chamber is the same throughout the circumference of the tooth at the level of the CEJ. The CEJ is the most consistent, repeatable landmark for locating the position of the pulp chamber.
- **Law of symmetry 1:** Except for maxillary molars, the orifices of the canals are equidistant from a line drawn in a mesiodistal direction through the pulp chamber floor.
- **Law of symmetry 2:** Except for the maxillary molars, the orifices of the canals lie on a line perpendicular to a line drawn in a mesiodistal direction across the center of the floor of the pulp chamber.
- **Law of color change:** The color of the pulp chamber floor is always darker than the walls.
- **Law of orifice location 1:** The orifices of the root canals are always located at the junction of the walls and the floor.
- **Law of orifice location 2:** The orifices of the root canals are located at the angle in the floor-wall junction.
- **Law of orifice location 3:** The orifices of the root canals are located at the terminus of the root developmental fusion lines.

## Root canal shape

The root canal can be divided into two components: *(1)* the main canal, which is mostly shaped by the use of endodontic instruments, and *(2)* the lateral components, comprised by isthmuses, accessory canals, and fins that are present in flattened and oval-shaped canals (Ordinola-Zapata et al 2018). In a longitudinal section, the root canal is usually broader buccolingually than in the mesiodistal plane. Based on their cross-sectional geometry, canals can also be classified as round, oval, long oval, flattened, or irregular in shape. Jou et al used the ratio of major and minor canal diameters to define an oval or long oval canal. An oval-shaped canal has an aspect ratio between 1 and 2, a long oval canal has a value higher than 2 but lower than 4, and a flattened canal has a value higher than 4 (Wu et al 2000). It has also been documented that the apical third is rounded or slightly oval in shape compared to the middle and coronal thirds. Although this is most commonly found in single-rooted teeth, molars with isthmuses and other irregularities may have varied cross-sectional shapes along the same root (Versiani et al 2013, Wu et al 2000).


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## Root canal configuration

The intricacy of the root canal anatomy illustrated in classic research by Hess and Zürcher revealed the need for a classification system to describe the root canal configurations. With the help of sectioning and radiographs, Weine et al were the first authors (in 1969) to classify the root canal anatomy into four distinct categories:

- **Type I:** A single canal extends from the pulp chamber to the apical foramen.
- **Type II:** Two separate canals leave the chamber but merge before the apex to form one apical foramen.
- **Type III:** Two separate canals leave the chamber and exit the root in separate apical foramina.
- **Type IV:** A single canal leaves the chamber but divides before the apex into two separate canals with separate apical foramina.

Later, Vertucci et al (in 1974) used the clearing process to establish a more thorough classification based on the examination of 200 maxillary second premolars. Vertucci was able to differentiate eight different configurations:

- **Type I:** A single canal extending from the pulp chamber to the foramen.
- **Type II:** Two separate canals leave the chamber but conjoin to exit in one foramen.
- **Type III:** One canal leaves the chamber, bifurcates within the root, then rejoins to exit in one foramen.
- **Type IV:** Two separate, distinct canals extend from the chamber to separate foramina.
- **Type V:** A single canal leaves the chamber, divides within the root, and exits in two foramina.
- **Type VI:** Two separate canals leave the chamber, merge within the root, and then redivide to exit in two distinct foramina.
- **Type VII:** One canal emanates from the chamber, divides and then rejoins within the root, then redivides into two distinct canals and foramina.
- **Type VIII:** Three separate and distinct canals extend from the chamber to the foramina.

The Weine and Vertucci configuration systems were based on the number of root canals that begin at the pulp chamber floor and open through an apical foramen. Vertucci's classification also included canals that arose along the course of the main canal. Versiani and Ordinola-Zapata used images acquired by microCT and expanded these classifications to describe at least 37 possible canal configurations that can be observed in a single root (Fig 17-2).

## Accessory canals

An accessory canal is any branch of the root canal that communicates with the periodontal ligament, while *lateral canals* are defined as accessory canals located at the coronal or middle root thirds (AAE). They are formed after a localized fragmentation of Hertwig's epithelial root sheath develops, leaving a small gap, or when blood vessels running from the dental sac through the dental papillae persist as collateral circulation. Accessory canals are able to connect the pulp canal space to the periodontium and can be situated at the root (lateral) or furcation level of the tooth (furcation canal). These interconnections are potential routes for bacteria and/or their byproducts from the necrotic root canal space to enter the periodontal ligament space and induce furcation involvement and lateral root lesions (Ricucci and Siqueira). Accessory canals (10 to 200 µm) are perceived to be smaller than the main canal but larger than dentinal tubules (0.5 to 3.2 µm) (Garberoglio and Brännström). Accessory canals are not normally visible in periapical images. Their presence can be suspected when a lateral root lesion is observed. They are detectable in CBCT slices if the voxel size is small enough to expose them. Only by utilizing proteolytic agents during the irrigation procedure can the content of lateral canals be neutralized (Ordinola-Zapata et al 2018, Tjäderhane).

Using the clearing approach, De Deus examined 1,140 teeth to determine the frequency of accessory canals and their locations. He demonstrated that there were accessory canals present in 27.4% of the sampled teeth, most frequently in the apical region (17%), followed by the middle (8.8%) and coronal (1.6%) thirds. Vertucci (1984) evaluated 2,400 teeth and found a reduced incidence of canal ramifications in the middle (11.4%) and coronal (6.3%) thirds compared to the apical level (73.5%).

In a morphologic analysis of 100 molars, previous authors found that over 80% of teeth had lateral and accessory foramina, with diameters ranging from 10 to 200 microns (Dammaschke et al). The diameter of the main apical foramen was two to three times larger than the diameter of the accessory canals (Green, Kuttler). It is possible that the size difference between the major apical foramen and the lateral and accessory foramina is one factor that contributes to the much higher prevalence of apical periodontitis compared to lateral periodontitis (Ricucci and Siqueira). In another study, Xu et al discovered that 86% of accessory canals were located in the apical third of 1,400 extracted teeth and that the tooth with the highest incidence of accessory canals was the maxillary second premolar (77%). In case of failure, numerous authors have proposed root-end resection of the apical 3 mm of the tooth followed by root-end preparation and filling in order to eliminate these accessory canals and their potentially toxic content (Song et al, Stropko).

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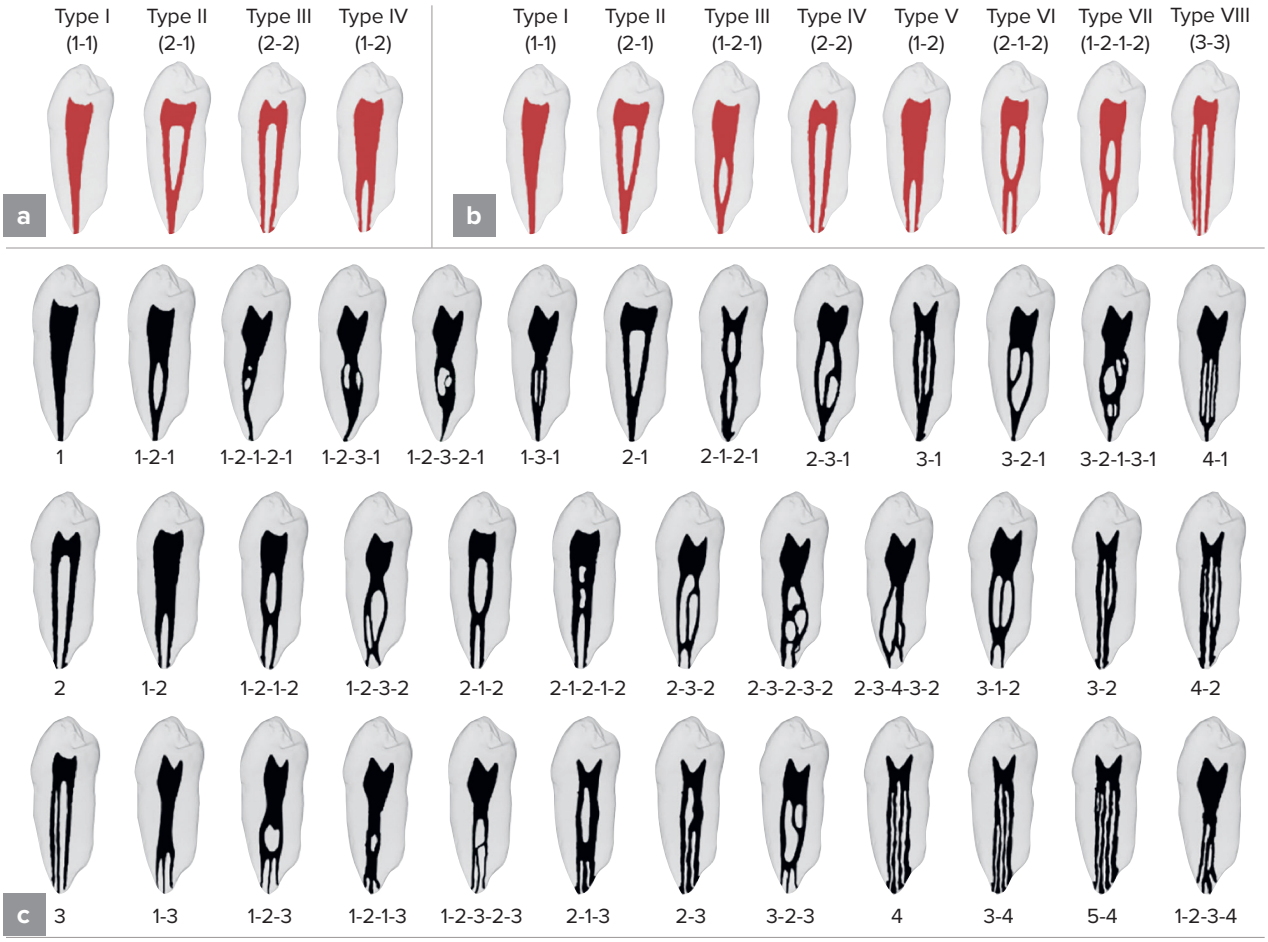


▲ **FIG 17-2** *(a and b)* Weine and Vertucci classification systems, respectively. *(c)* At least 37 canal configurations have now been described in the literature. (Modified from Versiani and Ordinola-Zapata.)

Furcation canals are accessory canals that connect the pulp chamber to the periodontal ligament in the furcation region of a multirooted tooth (Gutmann, Vertucci and Anthony). These canals originate from entrapment of periodontal vessels during fusion of the parts of the diaphragm, which becomes the floor of the pulp chamber (Cutright and Bhaskar). In some cases, furcation canals have been associated with primary endodontic lesions in the interradicular region of multirooted teeth. Gutmann studied the prevalence, location, and patency of accessory canals in the furcation region of permanent molars and reported their presence in 29.4% of mandibular molars and 27.4% of maxillary molars. In another study, Vertucci and Anthony observed the presence of foramina on both the pulp chamber floor and the furcation surface in 36% of maxillary first molars, 12% of maxillary second molars, 32% of mandibular first molars, and 24% of mandibular second molars.

## Isthmus

An *isthmus*, also called a *transverse anastomosis*, is defined as a narrow, ribbon-shaped communication between two root canals. Isthmuses can contain organic tissue, biofilms, or root filling material from previous treatment (Vertucci 2005, Weller et al). Hsu and Kim classified the configurations of isthmuses into five types (Fig 17-3):

- **Type I:** Two canals with no notable communication
- **Type II**: A hair-thin connection between the two main canals
- **Type III:** Differs from type II because of the presence of three canals instead of two
- **Type IV:** An isthmus with extended canals into the connection
- **Type V:** A true connection or wide corridor of tissue between the two main canals


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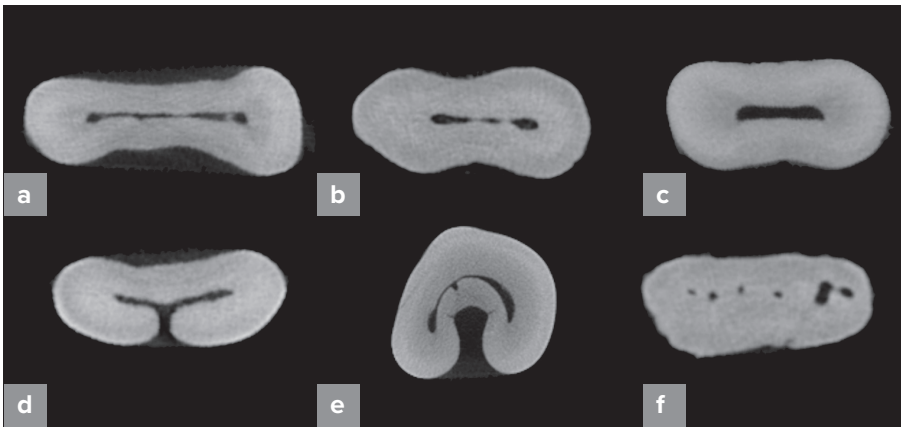



▶ **FIG 17-3** MicroCT transverse cross sections of posterior teeth showing the following: *(a)* a type II isthmus (hair connection); *(b)* a type III isthmus and the presence of a middle canal; *(c)* a true connection or wide corridor between the main canals; *(d)* an isthmus communicating the root canal system to the periodontal ligament through a lateral canal; *(e)* an isthmus connecting several canals in a fused root, leading to the formation of a C-shaped canal; and *(f)* an apical delta showing at least six portals of exit. (Reprinted with permission from Ordinola-Zapata et al 2018.)

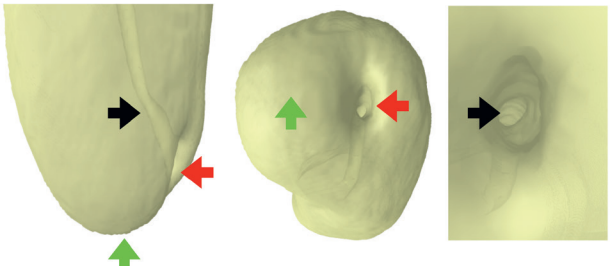
The prevalence of isthmuses is dependent on the type of tooth, the root level, and the patient's age. Estrela et al used CBCT to assess the frequency of root canal isthmuses in 1,400 adult permanent teeth. Isthmuses were found in 60.8% of the maxillary first molars, with the majority of them located in the mesiobuccal root (93.5%). In maxillary second molars, isthmuses were found in 46.5% of teeth (78.8% in the mesiobuccal roots). The highest frequency of isthmuses (87.9%) was found in mandibular first molars (57.8% in the mesial root and 42.2% in the distal root). In the mandibular second molars, the frequency of isthmuses was 66.3% (94.1% in mesial roots). Maxillary second premolars had more isthmuses (50.5%) than maxillary first premolars (18.8%), while the frequency was higher in mandibular first premolars (18.8%) than mandibular second premolars (3%). The frequencies of isthmuses that started at the middle or apical thirds were 50% (maxillary first molars), 42.2% (mandibular first molars), 82.3% (mandibular central incisors), and 55.1% (mandibular lateral incisors).

Laboratory studies indicated the inability to achieve complete debridement in the isthmus area, mostly due to accumulation of hard tissue debris during mechanical preparation of the main root canals (Chan et al, Keleş et al 2016, Leoni et al, Versiani et al 2016a). In addition, clinical studies have shown that unfilled isthmuses are typically detected following root-end resection procedures (von Arx). It is important to note that isthmuses need to be included in the cavity preparation of posterior teeth during the surgical treatment of posterior teeth (von Arx et al).

## Apical canal

The main root canal terminates in the major foramen (apical foramen), which commonly opens laterally on the root surface, 0.2 to 3.8 mm (on average) from the anatomical apex (Gutierrez and Aguayo). The apical foramen may coincide with the apex anywhere from 6.7% to 46% of the time, depending on the tooth group (AAE, Green, Mizutani et al, Pineda and Kuttler, Vertucci 1984). Green found that at 1 mm from the apex, the cross-sectional diameter of small canals (mesial of mandibular molars and mesiobuccal/distobuccal of maxillary molars) was between 200 and 400 microns. Large canals such as distal roots of mandibular molars or palatal canals of maxillary molars had a diameter between 400 and 700 microns.

The apical portion of the root canal with the narrowest diameter has been defined as the *apical constriction* or *minor foramen* (AAE). Kuttler discovered that the average distance between the foramen (apical foramen/major diameter) to the minor canal diameter (apical constriction) is 0.52 mm in individuals aged 18 to 25 years and 0.66 mm in those aged 55 and older. In the younger group, the average deviation of the apical foramen from the apical center was 68%, whereas in the older group it was 80%. The topography of the apical constriction is not constant (Dummer et al, Versiani et al 2013) and, when present, is usually located 0.5 to 1.5 mm from the center of the apical foramen (Vertucci 2005). From the apical constriction, the canal widens as it approaches the apical foramen (Fig 17-4).



▲ **FIG 17-4** MicroCT reconstruction of the apical third. The *arrows* identify important landmarks including the apex *(green)*, major foramen *(red)*, and apical constriction *(black)*.

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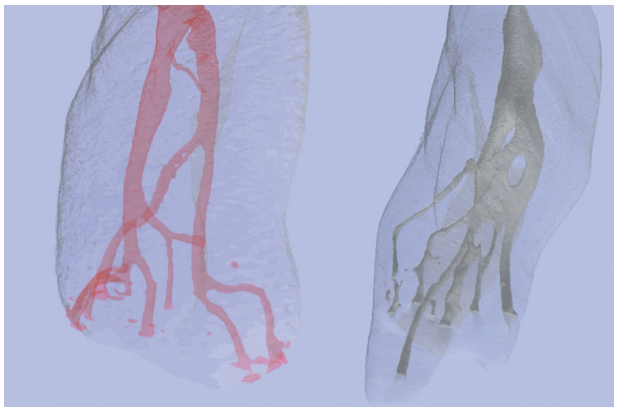

▲ **FIG 17-5** MicroCT reconstruction of two mandibular premolars presenting apical deltas. Multiple portals of exit can be observed in the last apical millimeters. These images show how complex disinfection of the apical third can become if bacteria gain access there. (Reprinted with permission from Chavez de Paz and Ordinola-Zapata.)

Another relevant variation of the root canal at or near the apex is an intricate network of ramifications known as the *apical delta*, which is defined as a morphology in which the main canal divides into multiple accessory canals (AAE) (Fig 17-5). In maxillary teeth, their frequency has been reported to range from 1% (central incisors) to 15.1% (second premolars), while in mandibular teeth, their frequency ranges from 5% (central incisors) to 14% (distal root of first molars) (Ricucci et al). Ricucci and Siqueira also reported a high prevalence of apical ramifications in 493 human teeth investigated under light microscopy, mostly at the apical portion of posterior teeth. Using microCT, Gao et al analyzed the apical morphology of 1,400 human teeth from a Chinese subpopulation. The authors reported a total of 634 apical branches (ranging from 3 to 18 per tooth), distributed in 6.3%, 8.8%, and 15.8% of anterior, premolar, and molar teeth, respectively. These branches started 0.62 to 5.08 mm from the apex, and in only 13% of these teeth (18 out of 136) was this vertical distance higher than 3 mm. This means that, in case of endodontic surgery, if 3 mm of the apical structure is resected, apical deltas with bacterial biofilms will be fully removed in 87% of cases (Siqueira et al). This circumstance requires careful inspection of the resected root surface using high magnification.

## Canal curvature

One of the elements that determines the complexity of treatment and increases the possibility of iatrogenic errors is the root canal's curvature. The preoperative diagnosis of root canal curvatures is of utmost importance to implement specific strategies that may require further clinical training (Versiani and Ordinola-Zapata). Multiple endodontic societies have produced case complexity evaluation criteria, and the root canal curvature is one of the characteristics considered (Faraj and Boutsioukis). Such evaluations are believed to be useful in deciding whether a patient should be sent to an endodontist or whether the case may be managed by a general practitioner (Hartmann et al).

Nearly all root canals are curved at the apical third, particularly in the buccolingual direction. Overall, curvatures may be categorized as a gradual curvature of the entire canal, a sharp curvature of the canal near the apex, or a gradual curvature of the canal with a straight apical ending. These variations in the canal curvature may not be evident on conventional radiographs (Vertucci 2005). Numerous methods have been proposed to determine root canal curvature (Nagy et al, Pruett et al, Schäfer et al, Schneider, Sonntag et al 2006, Sonntag et al 2005, Weine). Among them, Schneider's method has been the most widely used. The canal curvature is determined by drawing a line parallel to the long axis of the canal and then drawing a second line connecting the apical foramen to the point in the first line where the canal starts to leave the long axis of the tooth (Schneider). The angle formed by these two lines is the angle of curvature, and its degree is classified as straight (≤ 5 degrees), moderate (10 to 20 degrees), or severe (25 to 70 degrees) (Schneider).

The standard parallel projection offers only a 2D projection and makes the canals seem consistent in shape and taper. CBCT images can reveal missing canals and help determine the severity of root canal curvatures. In a clinical setting, two or three radiographic exposures at different horizontal angles may be needed to determine the root canal curvatures. Schäfer et al used radiographic exams to evaluate the degree of curvature of 1,163 root canals from all groups of teeth. The degree of curvature ranged from 0 to 75 degrees and from 0 to 69 degrees in clinical and proximal views, respectively. The highest degrees of curvature were observed in the clinical views of the mesiobuccal canal of maxillary molars and in the mesial canals of mandibular molars. Proximal curvature angles exceeded clinical view angles in several cases. Moreover, a secondary curvature (S-shaped canal) was observed in 12.3% and 23.3% of the maxillary and mandibular teeth, respectively. These findings revealed that canal curvatures are typically multiplanar in posterior teeth and that only angular views or CBCT analysis could identify the existence, direction, and degree of these variations (Fig 17-6).



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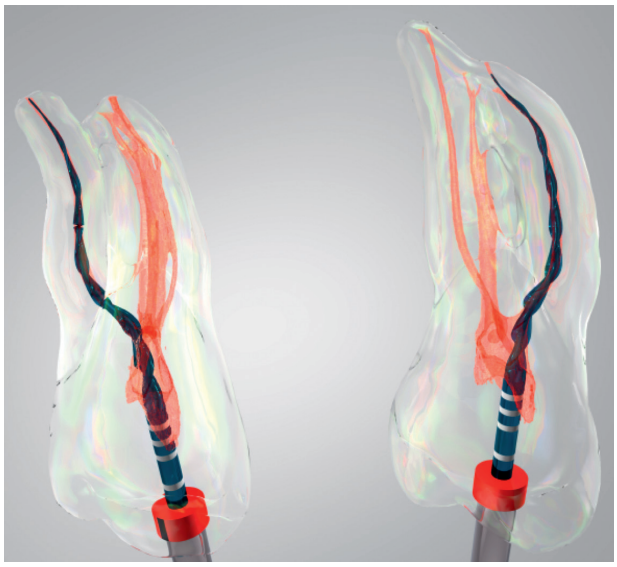

▲ **FIG 17-6** MicroCT 3D reconstructions showing the presence of multiplanar curvatures in the distobuccal and mesiobuccal roots of a maxillary molar. (Modified from Versiani et al 2023.)

## Anatomical Anomalies of Endodontic Significance

Root and root canal morphologic anomalies are common in the human dentition and can affect any tooth. Dental anomalies are formative defects due to genetic disturbances during the morphogenesis of teeth and may occur during the developmental stages or manifest once teeth are fully formed (Hargreaves and Cohen, Nanci and Ten Cate, Woelfel and Scheid). If abnormal teeth are not identified, an incorrect diagnosis and treatment strategy might result in iatrogenic damage. Tooth anomalies can also compromise the cleaning and shaping procedure (Vertucci 2005). The most common anomalies with impact on endodontic practice include taurodontism, dens invaginatus, dens evaginatus, extra roots (radix), and C-shaped canals.

### Taurodontism

Taurodontism is a dental morphologic variation in which the body of the tooth is enlarged and the root is reduced in length (AAE). A taurodont tooth presents a large pulp chamber with apical displacement of the pulpal floor and furcation of the roots. The etiology of taurodontism is unclear, but it also appears in certain genetic syndromes. It is thought to be caused by the failure of Hertwig's epithelial root sheath diaphragm to invaginate at the proper horizontal level, resulting in a tooth with normal dentin, short roots, elongated body, and enlarged pulp (Woelfel and Scheid, Nanci and Ten Cate). The teeth involved are almost invariably molars or, rarely, premolars. Taurodontism can be unilateral or bilateral and can affect single or multiple teeth. It was previously classified by Shaw according to its severity as normal (cynodontism), least pronounced (hypotaurodontism), moderate (mesotaurodontism), and most severe (hypertaurodontism). Clinically, the crowns of these teeth usually have normal characteristics and therefore diagnosis is entirely radiologic (Sert and Bayrl). Due to the complexity of the root canal anatomy and the proximity of the orifices to the root apex, complete filling of the apical canal system in taurodontism is challenging.

### Dens invaginatus

Dens invaginatus is a developmental defect resulting from invagination in the surface of the tooth crown before calcification has occurred (Nanci and Ten Cate). Clinically, it may appear as an accentuation of the lingual pit in anterior teeth. In its more severe form, it gives the radiographic appearance of a tooth within a tooth (Fig 17-7), hence the colloquial term *dens in dente* (AAE). Its etiology is controversial and remains unclear. The affected teeth radiographically show an infolding of enamel and dentin that may extend deep into the pulp cavity and into the root, sometimes even reaching the root apex (Hülsmann). The invagination also allows the entry of irritants into an area that is separated from pulpal tissue by only a thin layer of enamel and dentin, thereby presenting a predisposition for the development of dental caries (Hülsmann).

▲ **FIG 17-7** Dens invaginatus in a maxillary lateral incisor.

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The variability of the root canal system configuration in the case of dens invaginatus was classified by Oehlers into three types:

- **Type I:** The invagination is confined to the crown and does not extend beyond the CEJ.
- **Type II:** The invagination extends past the CEJ and does not involve the periradicular tissues but may communicate with the dental pulp.
- **Type III:** The invagination extends beyond the CEJ and may present a second apical foramen, with no immediate communication with the pulp.

In the literature, the reported prevalence of this anomaly varies from 0.25% to 10%. While it may occur in any tooth, the most affected teeth are permanent maxillary lateral incisors (Hülsmann, Oehlers).

## Dens evaginatus

Dens evaginatus is an anomalous outgrowth of tooth structure resulting from the folding of the inner enamel epithelium into the stellate reticulum, with the projection of structure exhibiting enamel, dentin, and pulp tissue (AAE). It most frequently arises from the occlusal surface of involved posterior teeth, mainly maxillary and mandibular premolars, and from the lingual surface of associated anterior teeth (called *talon cusps* when in this location) (Levitan and Himel, Oehlers et al). Its etiology remains unclear, but this anomaly predominantly occurs in people of Asian descent ,with varying prevalence estimates reported at 0.5% (Kocsis et al) to 15% (Merrill). The presence of pulp within the cusp-like tubercle has great clinical significance. Because the tubercle may extend above the occlusal surface, malocclusion or attrition with the opposing tooth may cause abnormal wear or fracture of the tubercle, thus exposing the pulp and potentially leading to subsequent pulpal inflammation or infection (Levitan and Himel). If pulp necrosis occurs at a young age, the affected tooth may be prone to incomplete root development.

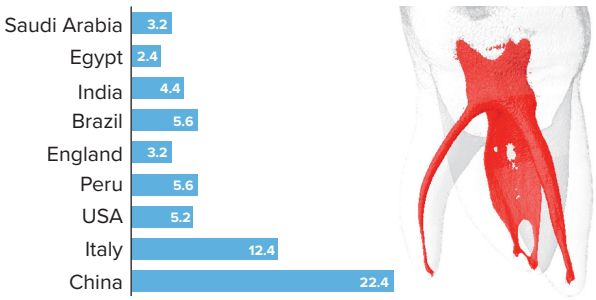
## Radix

*Radix* is a Latin word for *root* and refers to additional roots of teeth, mostly molars. In four-rooted maxillary molars, the palatal part of the root complex is made up of two macrostructures located mesially and distally, which are in principle cone-shaped and either separate or nonseparate in relation to each other. If the mesial of the two palatal root structures has direct affinity to the mesiolingual part of the crown, which is more pronounced, the mesial root structure is identified as radix mesiolingualis, whereas the distal structure is identical with the palatal root component. If the distal of the two palatal root structures has direct affinity with the distopalatal part of the crown, the distal root structure is identified as radix distolingualis, whereas the mesial structure is identical with the palatal root component (Carlsen and Alexandersen).

In mandibular molars, additional roots have been identified as radix entomolaris and radix paramolaris. *Radix entomolaris* has been defined as a supernumerary root on a mandibular molar located distolingually, whereas *radix paramolaris* is an extra root located mesiobuccally (AAE). The occurrence of certain anatomical variations has been linked to particular ethnic groups, including individuals of Chinese, Inuit, or Native American descent (Calberson et al, De Moor et al) (Fig 17-8). The main endodontic concern with the distolingual root is its abrupt curvature at the apical third, which could increase the risk of instrument separation. Radix paramolaris is a very rare structure, and its prevalence was found to be 0%, 0.5%, and 2.0% for the mandibular first, second, and third molars, respectively, whereas radix entomolaris occurs with a higher frequency, ranging from 0.2% to 32% (Turner, Carlsen and Alexandersen). These anatomical variations present challenges to treatment because of their orifice inclination and root canal curvature.

## C-shaped canals

This anatomical variation is so named for the root and root canal cross-sectional shape of the capital letter "C." Its main anatomical feature is the presence of one or more isthmuses connecting individual canals, which can change the cross-sectional and 3D canal shape along the root (Melton et al) Typically, this configuration is found in teeth with fused roots either on the buccal or lingual aspect and results from the failure of Hertwig's epithelial




▲ **FIG 17-8** The prevalence of radix entomolaris (%) in different populations. (Modified from Martins et al 2022; 3D reconstruction modified from Rodrigues et al.)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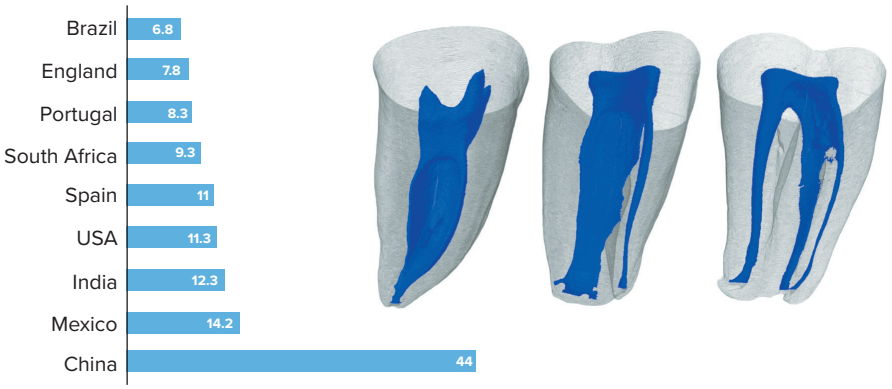
▶ **FIG 17-9** Mandibular second molar with a C-shaped root. Observe the presence of fused roots with a deep radicular groove. The pulp chamber access cavities reveal three different configurations, including a continuous C-shaped orifice, an isolated mesiolingual canal with an isthmus connecting the mesiobuccal and distal canals, and a variation showing independent root canal orifices.




▶ **FIG 17-10** The prevalence of C-shaped canals in mandibular second molars (%) in different populations and 3D reconstructions demonstrating typical, asymmetric, and symmetric C-shaped canals. (Modified from Martins et al 2019; 3D reconstructions modified from Amoroso-Silva et al.)

root sheath to develop or fuse in the furcation area during tooth development (Jafarzadeh and Wu). In such teeth, the pulp chamber floor is frequently situated deeply and may assume an unusual anatomical appearance. Below the orifice level, the root structure of a C-shaped tooth can harbor a wide range of anatomical variations, which make it a challenge with regard to disinfection (Jafarzadeh and Wu). This variation may occur in different types of teeth including mandibular molars, premolars, and maxillary second molars (Ordinola-Zapata et al 2017); however, it is most commonly found in mandibular second molars (Fig 17-9), with a reported prevalence ranging from 2.7% to 44.5% (Versiani et al 2018b). There is significant ethnic variation in the frequency of C-shaped molar teeth, which are much more common in Asian than in Caucasian populations (Fig 17-10) (Martins et al 2019b).

In 1991, Melton et al proposed a classification for C-shaped canals based on histologic slides:

- **Category 1:** A continuous C-shaped canal running from the pulp chamber to the apex
- **Category 2:** A semicolon-shaped orifice in which dentin separates a main C-shaped canal from one mesial distinct canal
- **Category 3:** Two or more discrete and separate canals

The C-shaped canal anatomy has also been reported in third molars, lateral incisors, mandibular first premolars, mandibular first molars, and maxillary first and second molars (Martins et al 2019b). In maxillary molars, C-shaped canals are uncommon (Vertucci 2005). Newton and McDonald were the first to describe a C-shaped configuration caused by the confluence of the distobuccal

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**TABLE 17-1 Morphologic aspects of the root and root canal anatomy of mandibular anterior teeth**

| | Incisors | Canine |
|---|---|---|
| Overall length | Central: 20.8 mm (16.9–26.7 mm)<br>Lateral: 22.1 mm (18.5–26.6 mm) | 25.9 mm (16.1–34.5 mm) |
| Root length | Central: 12.6 mm (7.7–17.9 mm)<br>Lateral: 13.5 mm (9.4–18.1 mm) | 15.9 mm (9.5–22.2 mm) |
| Number of roots | Central: 1 (100%)<br>Lateral: 1 (99.92%), 2 (0.08%) | 1 (98.57%), 2 (1.43%) |
| Number of canals | Central: 1 (86.5%), 2 (14.4%), other (0.1%)<br>Lateral: 1 (79.7%), 2 (20.2%), other (0.1%) | 1 (92.4%), 2 (7.3%), other (0.3%) |
| Accessory canals | Central: 0% to 20% (coronal: 3%; middle: 12%; apical: 85%)<br>Lateral: 0.9% to 18% (coronal: 2%; middle: 15%; apical: 83%) | 4.5% to 30% (coronal: 4%; middle: 16%; apical: 80%) |

Data from Versiani et al 2018a.

and palatal orifices in a maxillary first molar, comparable to the C-shaped configuration observed in mandibular molars. Ordinola-Zapata et al (2017) found that the prevalence of C-shaped root canal systems in 100 maxillary molars with fused roots was 22%. As in mandibular molars, C-shaped canal systems in mandibular first premolars vary among different ethnic groups, with their prevalence being reported to range from 1% to 18% (Martins et al 2019b). In mandibular premolars, this configuration has been strongly associated with the Vertucci type V configuration, with the presence of a groove or concavity on the external root surface that usually begins 3 mm from the CEJ and presents frequently on the proximal lingual area of the middle root, not always extending to the root apex (Boschetti et al, Ordinola-Zapata et al 2013, Ordinola-Zapata et al 2015).

## Root Canal Anatomy

This section describes the most relevant morphologic aspects of the root and root canal anatomy of all groups of teeth, depicting in tables data extracted from current and classical studies and using images acquired from nondestructive microCT technology for illustration.

### Mandibular teeth

#### Anterior teeth

The mandibular central and lateral incisors have smaller mesiodistal dimensions than any of the other teeth. These teeth are similar in form and have smooth crown surfaces that show few traces of developmental lines. Although it is the smallest tooth in the mouth, the labiolingual root diameter of the mandibular central incisor is significantly larger than the other interproximal dimensions (Nelson and Ash, Versiani et al 2018a). This tooth usually has one canal (75%). Two ribbon-shaped canals may be found, but not very frequently (15% and 20% of central and lateral incisors, respectively). The pulp horns are well developed in this tooth group. The mandibular lateral incisor tends to be slightly larger than the mandibular central incisor in all dimensions, including the pulp chamber. The pulp canal may taper gently from the apex or narrow abruptly in the last 3 to 4 mm of the root canal (Nelson and Ash). Ideal straight-line access has been determined by Mauger et al to be at the incisal edge in 72% of cases. In their study, as the wear of the incisal edge increased, the ideal access moved from the facial aspect toward the incisal border (Mauger et al).

The mandibular canine root may be as long as that of the maxillary canine, but usually it is somewhat shorter. The pulp cavity of the mandibular canine tends to be a little shorter than that of the maxillary canine. The variation of bifurcated roots in the mandibular canine is not rare, and it is also not uncommon to find two roots or at least two canals. Because the presence of two canals cannot be easily detected radiographically, their presence must be ruled out clinically as well. Some mandibular canines demonstrate an abrupt narrowing of the pulp cavity when passing from the pulp chamber to the pulp canal (Versiani et al 2018a). Other mandibular canine teeth demonstrate an abrupt narrowing of the pulp canal in the apical region (Nelson and Ash) (Table 17-1).


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**TABLE 17-2 Morphologic aspects of the root and root canal anatomy of mandibular premolars**

| | First premolar | Second premolar |
|---|---|---|
| Overall length | 22.4 mm (17.0–28.5 mm) | 22.1 mm (16.8–28.1 mm) |
| Root length | 14.4 mm (9.7–20.2 mm) | 14.7 mm (9.2–21.2 mm) |
| Number of roots | 1 (97.5%), 2 (2.5%) | 1 (98.5%), 2 (1.5%) |
| Number of canals | 1 (71.3%), 2 (27.9%), 3 (0.1%), other (0.7%) | 1 (84.7%), 2 (15.05%), 3 (0.05%), other (0.2%) |
| Accessory canals | 8.8% to 44.3% (coronal: 4.3%; middle: 16.1%; apical: 78.9%) | 4% to 48.3% (coronal: 3.2%; middle: 16.4%; apical: 80.1%) |

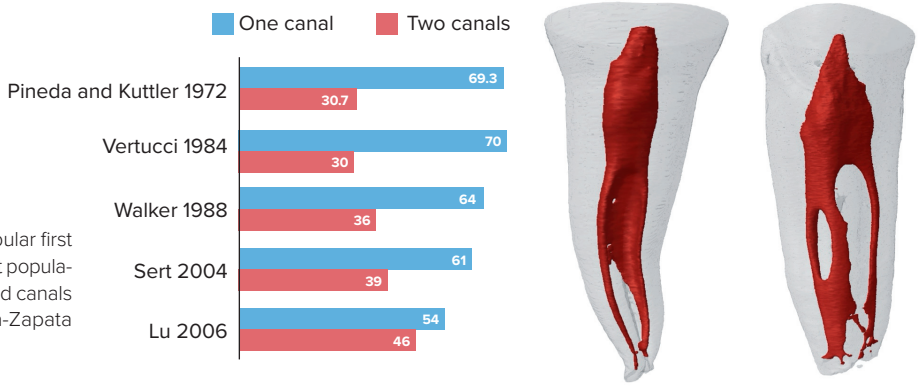
Data from Versiani et al 2018a.

It is crucial to note that each of these teeth is highly likely to have a second lingual root canal. The prevalence of Vertucci type I root canal configuration varies from approximately 60% (Shemesh et al 2018) to above 90% (Zhengyan et al) for both incisors and appears to be highly correlated with demographic factors, such as ethnicity; multiple studies report a higher prevalence of the single root canal configuration in Chinese subpopulations (Lin et al, Martins et al 2018b). The results for the mandibular canine appear to be more homogeneous, with the majority of studies reporting proportions around 90% (da Silva et al, Martins et al 2018b, Zhengyan et al). When the presence of a lingual canal is noted, the type III configuration is the most prevalent for the incisors (Shemesh et al 2018), while a mix of types III and V can be observed in the canines, a condition that may be related to the possibility of a two-rooted morphology in this tooth (da Silva et al, Martins et al 2018b).

### Premolars

The mandibular first premolar is always the smaller of the two mandibular premolars, whereas the opposite is true in maxillary premolars. Most of these teeth have one canal, but the presence of two or three canals has been described. The pulp chamber is usually very large, and the pulp cavity may taper gently toward the apex or abruptly as the root canal starts (Versiani et al 2018a). The root of the mandibular first premolar usually shows a deep developmental groove that has been associated with complex anatomical features including C-shaped (Baisden et al) and extra root canals (Nelson and Ash). The mandibular second premolar usually has three well-formed cusps: one large buccal cusp and two smaller lingual cusps. However, two-cusped forms of this tooth are also fairly common. It usually has one root and one canal that may be curved, usually in the distal direction. The pulp horns are prominent, and the pulp chamber and root canal gently taper toward the apex (Versiani et al 2018a). The single root of the second premolar is larger and longer than that of the first premolar. The root is seldom, if ever, bifurcated, although some specimens show a deep developmental groove buccally (Nelson and Ash) (Table 17-2).

The current literature shows that these teeth mostly present a Vertucci type I configuration, with a prevalence ranging from around 70% (Arslan et al) to 80% (Yu et al) for the first premolar and of almost 90% for the second premolar (Arslan et al) (Fig 17-11). The prevalence of




▶ **FIG 17-11** The prevalence of mandibular first premolars with two canals (%) in different populations and 3D reconstruction of C-shaped canals in premolars. (Modified from Ordinola-Zapata et al 2015.)

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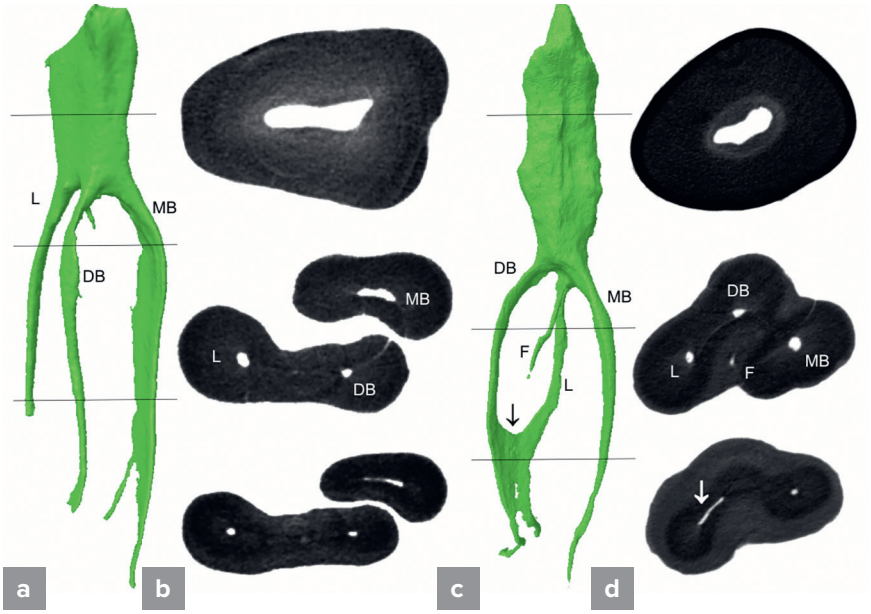


◀ **FIG 17-12** Representative 3D models and cross sections of two mandibular premolars with three canals. *(a and b)* Division of the main root canal into three separate canals at the middle third. *(c and d)* Fusion of distobuccal and lingual canals at the apical third *(arrows)*. Observe the presence of a furcation canal. (Reprinted with permission from Ordinola-Zapata et al 2013.)

a second lingual canal is highly correlated with gender, geographic location, and age (Martins et al 2021), and the most common configuration is Vertucci type V (Arslan et al, Martins et al 2018b). Less common configurations of great relevance, such as three root canals or C-shaped morphologies, account for proportions in the range of 1.1% to 10.9% (Baisden et al, Martins et al 2017, Martins et al 2019b, Wu et al 2017) (Fig 17-12). When three canals are present in a mandibular premolar, the specimen usually has one root with a main canal that is divided into mesiobuccal, distobuccal, and lingual canals at the furcation level. The configuration of the pulp chamber is mostly triangle-shaped (Ordinola-Zapata et al 2013).

### Molars

The mandibular first molar is usually the largest tooth in the mandibular arch. It has five well-developed cusps and two well-developed roots (one mesial and one distal), which are very broad buccolingually. These roots are widely separated at the apices. The buccolingual cross section of the mandibular first molar demonstrates a large pulp chamber that may extend well down into the root formation. The mesial root usually has a more complicated root canal system because of the presence of two canals. The distal root usually has one large canal, but two canals are often present. Occasionally, a fourth canal is present that has its own separate root.

Normally, the mandibular second molar has four well-developed cusps (two buccal and two lingual) of nearly equal size. The tooth has two well-developed roots, one mesial and one distal. These roots are broad buccolingually, but they are not as broad as those of the first molar, nor are they as widely separated. The buccolingual section of the mandibular second molar demonstrates a pulp chamber and pulp canals that tend to be more variable and complex than those found in the mandibular first molar (Nelson and Ash) (Table 17-3).

A wide variation has been reported in the root canal configuration of this group of teeth depending on the root morphology. Considering the most typical two-rooted configuration, Vertucci types II and IV account for 80% to 90% of all cases in the first molar mesial root, usually with an equal distribution of both types, although some studies report higher type IV proportions (Kim et al 2013, Martins et al 2019a, Perez-Heredia et al); in the second molar, the type I configuration may reach 10% (Kim et al 2016), with Vertucci types II and IV accounting for 80% of the cases (Martins et al 2018b) (Figs 17-13 and 17-14). As for the distal root of the first molar, Vertucci type I is the most common configuration, with a prevalence ranging from 63% (Wang et al) to 88% (Silva et al). When a second root canal is present, types II, III, and IV are the most common configurations in the mandibular first molar (Fig 17-15). In the second molar, the Vertucci type I configuration is observed in more than 90% of cases (Perez-Heredia et al).

Relevant variations exist in these teeth that are highly correlated with the root configuration. Among the most important variations are the presence of a radix paramolaris, with a proportion around 1% (Shemesh et al 2015), or


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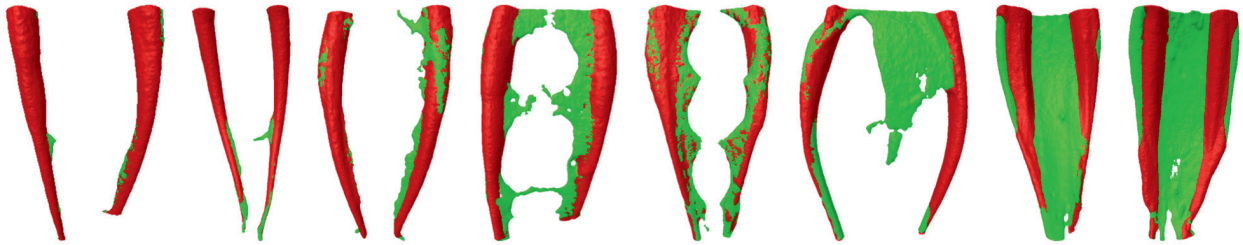
**TABLE 17-3 Morphologic aspects of the root and root canal anatomy of mandibular molars**

| | First molar | Second molar |
|---|---|---|
| Overall length | 20.9 mm (17.0–27.7 mm) | 20.6 mm (15.5–25.5 mm) |
| Root length | Mesial: 14.0 mm (10.6–20.0 mm)<br>Distal: 13.0 mm (8.1–17.7 mm) | Mesial: 13.9 mm (9.3–18.3 mm)<br>Distal: 13.0 mm (8.5–18.3 mm) |
| Number of roots | 2 (86.9%), 3 (12.5%), 1 (0.55%), 4 (0.05%) | 2 (78.6%), 1 (19%), 3 (2.2%), 4 (0.2%) |
| Number of canals | Mesial: 1 (2.37%), 2 (96.59%), 3 (0.03%), other (1.01%)<br>Distal: 1 (70.3%), 2 (29.56%), other (0.14%) | Mesial: 2 (87.1%), 1 (12.5%)<br>Distal: 1 (92.56%), 2 (7.44%) |
| Accessory canals | Mesial: 45% (coronal: 10.4%; middle: 12.2%; apical: 54.4%)<br>Distal: 30% (coronal: 8.7%; middle: 10.4%; apical: 57.9%) | Mesial: 49% (coronal: 10.1%; middle: 13.1%; apical: 65.8%)<br>Distal: 34% (coronal: 9.1%; middle: 11.6%; apical: 68.3%) |

Data from Versiani et al 2018a.

◀ **FIG 17-13** Middle mesial canals in the mesial root of mandibular molars. (Reprinted with permission from Versiani et al 2016.)



▲ **FIG 17-14** 3D microCT models of the mesial root canal system of eight mandibular molars presenting regular and irregular root canals as well as canals connected by an isthmus. Observe that as the complexity of the root canal system increases, the amount of nonprepared canal surface areas *(green)* also increases. (Reprinted with permission from Versiani et al 2015.)

a radix entomolaris, with a prevalence varying from 5% to 22% in the first molar (Martins et al 2022). In both cases, the radix was associated with a single root canal configuration (Fig 17-16). Additionally, the prevalence of C-shaped morphologies of the second molar ranges between 8% and 40% according to ethnic group (Martins et al 2019b).

**The middle mesial canal.** The middle mesial canal (MMC) has been defined as an extra canal located between the mesiobuccal and mesiolingual canals of the mesial root of mandibular molars. While its existence has been recognized for more than 100 years (Barker et al, Fischer, Lenhossèk, Vertucci et al), it was only in 1981 that a comprehensive in vivo study described its incidence and clinical management (Pomeranz et al). According to Pomeranz et al, the MMC canal presents three different configurations: *(1)* independent—three independent

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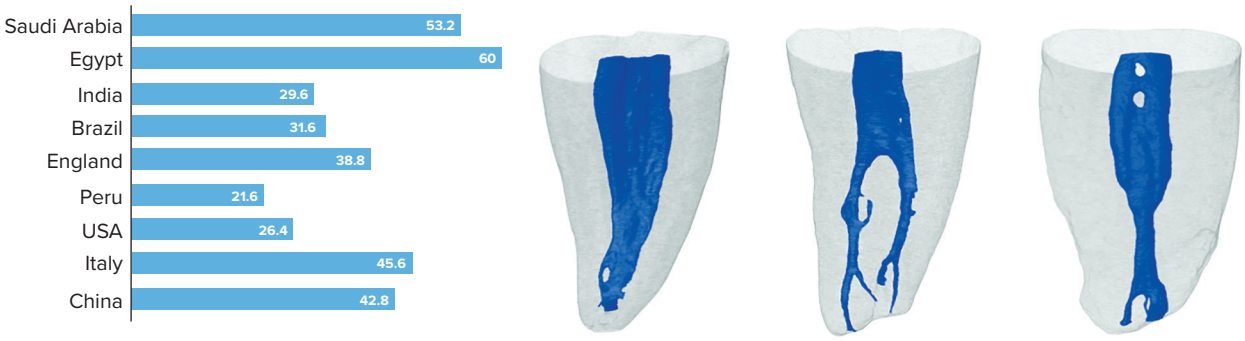
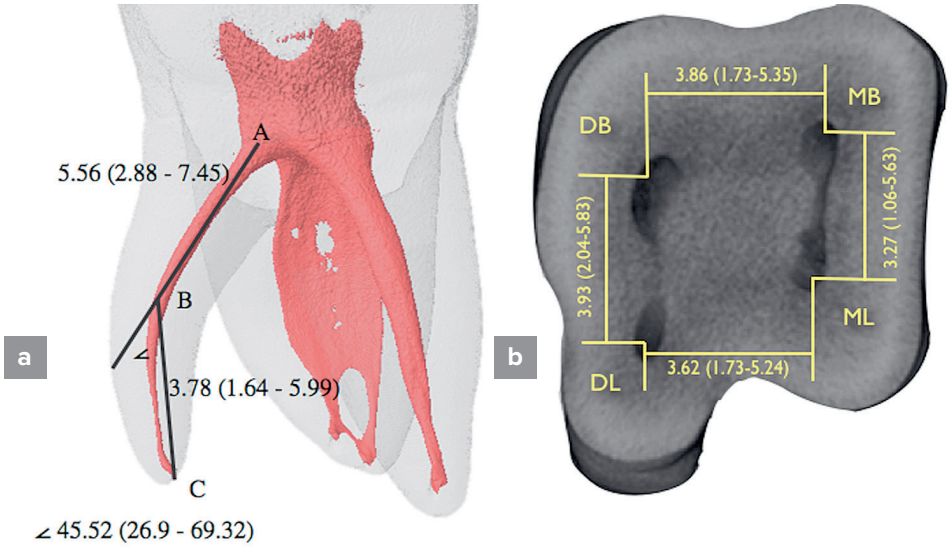



▲ **FIG 17-15** The prevalence of two canals in the distal roots of mandibular molars (%) in different populations. (Modified from Martins et al 2022; 3D reconstruction modified from Filpo-Perez et al.)




◀ **FIG 17-16** *(a)* The median and range values of the distance between the pulp chamber floor and the beginning of the curvature (A to B) and the distance between the curvature and the apical foramen (B to C) in the distolingual root. The < indicates the median and range values of the angle of curvature in the distolingual root. *(b)* The median and range values of the interorifice distances at 1 mm from the pulp chamber floor. (Reprinted with permission from Rodrigues et al.)

canals extend from the pulp chamber to the apex anatomy; *(2)* fin—in the coronal third, the MMC orifice is connected to the mesiobuccal and/or mesiolingual orifice(s) by a groove, but the mesial canals leave the root in three separate foramina; and *(3)* confluent—the MMC leaves the pulp chamber, whether separate from the other mesial canals or not, and joins the mesiobuccal canal and/or mesiolingual canal by transverse anastomosis, intercanal connection, or isthmus during its trajectory to the apical foramen. In the literature, this anatomical variation has been found at a frequency ranging from 0.26% (Kim et al 2013) to 2.6% (Fabra-Campos et al) to 46.15% (Azim et al). The confluent anatomy was the most common configuration type reported in a microCT study (Versiani et al 2016b). The dissimilar prevalence of MMC in these studies have been explained through diversity in sample size, study design, and/or racial factors, as well as patient age (Azim et al).

It has been postulated that during the growth of the root, the accumulation of secondary dentin forms dentinal vertical partitions inside the root canal cavity, thus creating three independent mesial root canals. It is also possible that dentin is partially deposited along the length of the root, leading to the formation of three canals that converge in either two foramina or one foramen. These canals may have transverse connections, thus forming a complex network of root canals (Peiris et al, Pomeranz et al). Numerous authors support the view that the MMC canal can be easily located in young patients but progressively decreases in incidence with age (Azim et al, Fabra-Campos, Goel et al, Kim et al 2013, Nosrat et al, Pomeranz et al).


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**TABLE 17-4 Morphologic aspects of the root and root canal anatomy of maxillary anterior teeth**

| | Central incisor | Lateral incisor | Canine |
|---|---|---|---|
| Overall length | 23.6 mm (16.5–32.6 mm) | 22.5 mm (17.7–28.9 mm) | 26.4 mm (20.0–38.4 mm) |
| Root length | 13.0 mm (6.3–20.3 mm) | 13.4 mm (9.6–19.4 mm) | 16.5 mm (10.8–28.5 mm) |
| Number of roots | 1 (99.94%), 2 (0.06%) | 1 (99.94%), 2 (0.06%) | 1 (100%) |
| Number of canals | 1 (99.2%), 2 (0.8%) | 1 (98.5%), 2 (1.5%) | 1 (97%), 2 (3%) |
| Accessory canals | 18.9% to 42.6% (coronal: 1%; middle: 6%; apical: 93%) | 5.5% to 26% (coronal: 1%; middle: 8%; apical: 91%) | 3.4% to 30% (coronal: 0%; middle: 10%; apical: 90%) |

Data from Versiani et al 2018a.

**Danger zone.** The term "danger zone" was introduced by Abou-Rass et al in 1980 to describe a specific region of the tooth's root that is susceptible to strip perforation. This could happen in cases of excessive removal of dentin during mechanical preparation. In mandibular molars, the danger zone has been usually associated with the distal aspect of the mesial root (furcation area) (Garcia Filho et al, Keleş et al 2020, Leite Pinto et al, Olivier et al, Sant'Anna Junior et al, Tabrizizadeh et al). In the literature, the smallest dentin thickness along the distal surfaces of mesial roots of mandibular molars has been reported to be 0.67 mm (De-Deus et al 2019), 0.79 mm (Garcia Filho et al), 0.94 mm (Lim and Stock), 1.05 mm (Akhlaghi et al), 1.08 mm (Kessler et al), 1.2 mm (Berutti and Fedon), and 1.3 mm (Tabrizizadeh et al).

Sauáia et al revealed that long mesial roots of mandibular molars present the thinnest walls and deepest distal concavities compared to short roots, while De-Deus et al (2021) reported that the length of the tooth/root, the distance of the mesiobuccal and mesiolingual canal orifices, and the depth of the mesial/distal groove of mandibular first molars could be predictive factors for the position, thickness, and direction of the danger zone. To prevent strip perforations, coronal preflaring needs to be restricted and directed at the outer and thicker aspect of the dentinal walls.

The concept of the danger zone was recently revisited by De-Deus et al (2019), who demonstrated that in most of the evaluated mandibular molars, the danger zone was located 4 to 7 mm (mean of 4.37 ± 1.68 mm) below the furcation area.

## Maxillary teeth

### Anterior teeth

The maxillary central incisors are centered in the maxilla, one on either side of the midline, with the mesial surface of each in contact with the mesial surface of the other. The pulp cavity follows the general outline of the crown and root. In this way, the pulp chamber is very narrow in the incisal region and wider in the mesiodistal dimension than in the labiolingual dimension (Nelson and Ash). The maxillary lateral incisor supplements the central incisor in function, and the crowns bear a close resemblance. However, the lateral incisor is smaller in all dimensions except root length. The pulp chamber is narrow in the incisal region and may become very wide at the cervical level of the tooth, while pulp horns are usually prominent (Nelson and Ash). Maxillary anterior teeth are usually exposed to dental trauma, and thus pulp canal obliteration or calcification of the pulp chamber is frequently seen.

The maxillary canines are the longest teeth in the mouth. The crowns are usually as long as those of the maxillary central incisors, and the single roots are longer than those of any of the other teeth. Therefore, the maxillary canine has the largest labiolingual root dimension of any tooth, and because the pulp cavity corresponds closely to the outline of the tooth, the size of the pulp chamber may also be the largest in the mouth (Nelson and Ash).

The Vertucci type I root canal configuration has been reported in almost 100% of maxillary incisors and canines (Martins et al 2019a) (Table 17-4). The few exceptions to this come from developmental anomalies such as dens invaginatus, especially in maxillary lateral incisors.

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**TABLE 17-5 Morphologic aspects of the root and root canal anatomy of maxillary premolars**

| | First premolar | Second premolar |
|---|---|---|
| Overall length | 21.5 mm (15.5–28.9 mm) | 21.2 mm (15.2–28.4 mm) |
| Root length | 13.4 mm (8.3–19.0 mm) | 14.0 mm (8.0–20.6 mm) |
| Number of roots | 2 (55.3%), 1 (43.1%), 3 (1.6%) | 1 (86.2%), 2 (13.5%), 3 (0.3%) |
| Number of canals | 2 (77.3%), 1 (20.1%), 3 (1.2%), other (1.3%) | 2 (56.7%), 1 (42.7%), 3 (0.4%), other (0.3%) |
| Accessory canals | 17.8% to 49.5% (coronal: 4.7%; middle: 10.3%; apical: 74%) | 12.9% to 59.5% (coronal: 4%; middle: 16.2%; apical: 78.2%) |

Data from Versiani et al 2018a.

### Premolars

The maxillary first premolar has two cusps, a buccal and a lingual, and each is sharply defined. The buccal cusp is usually about 1 mm longer than the lingual cusp, and because of that, the pulp horn usually extends further occlusally under the buccal cusp than the lingual cusp. The maxillary first premolar may have two well-developed roots, two root projections that are not fully separated, or one broad root (Nelson and Ash). The majority of maxillary first premolars have two root canals, but a small percentage of teeth may have three roots and three canals that may at times be difficult to see or almost undetectable radiographically. The pulp chamber floor is below the cervical level of all the variations found in this tooth group (Nelson and Ash). A palatal groove has been reported in up to 83% of maxillary first premolars (Lammertyn et al). The groove is found in the palatal side of the buccal root, and overinstrumentation during shaping or post space procedures can lead to strip perforations (Lammertyn et al).

The maxillary second premolar supplements the maxillary first premolar in function and closely resembles it in shape. The maxillary second premolar may have a crown that is noticeably smaller cervico-occlusally and also mesiodistally (Versiani et al 2018a). However, it may also be larger in those dimensions. Usually the root of the second premolar is as long as, if not a millimeter or so longer than, that of the first premolar. Most maxillary second premolars have only one root and canal. Two roots are possible, although two canals within a single root may also be found. The pulp chamber and root canal are very broad in the buccolingual aspect of teeth with single canals (Nelson and Ash).

Vertucci type IV has been reported as the most prevalent root canal configuration for the maxillary first premolar, with a proportion varying from 42% (Li et al) to 68% (Bürklein et al), followed by types II and I, both with percentages between 10% and 20% (Martins et al 2019a). For the second premolar, the higher prevalence is for type I configurations, with percentages that may reach 50% of cases (Li et al) or above, followed by type II. However, it is worthwhile to highlight that the variability of the root canal configuration of both maxillary premolars is so high that any Vertucci type can be found and expected, including complex morphologies with three root canals, with a proportion as high as 3% (Abella et al) (Table 17-5).

### Molars

The maxillary first molar is normally the largest tooth in the maxillary arch. The complexity of maxillary first molars may surpass that of all other teeth within the human dentition (Versiani et al 2018a). The crown of this tooth has its largest diameter in the buccolingual direction. It has four well-developed functioning cusps and one supplemental cusp (the cusp of Carabelli). Molar access should be centered mesiodistally on the occlusal surface and need not extend to the mesial marginal ridge (Wilcox et al). The maxillary first molar usually has three roots and three or four canals. The palatal root usually has the largest dimensions, followed by the mesiobuccal and distobuccal roots, respectively. The mesiobuccal root normally presents an accessory canal commonly called *mesiolingual* or *MB2*, which usually is the smallest of all the canals in this tooth (Nelson and Ash). More extensive use of the operatory microscope has revealed that not only a fourth canal but other additional canals also exist.

The maxillary second molar supplements the first molar in function. The propensity for root fusion is more common in the maxillary second molar than in the first molar (Versiani et al 2018a). Most often maxillary second molars possess three roots and three canals. The mesiobuccal root of the second molar is not as complex as that of the first molar. Four roots have also been described in some maxillary second molars; the roots are usually straight with similar lengths, and the root canals are


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**TABLE 17-6 Morphologic aspects of the root and root canal anatomy of maxillary molars**

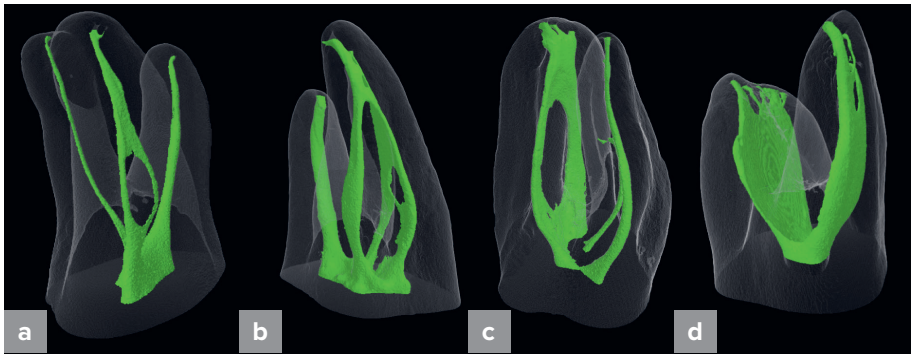
| | First molar | Second molar |
|---|---|---|
| Overall length | 20.1 mm (17.0–27.4 mm) | 20.0 mm (16.0–26.2 mm) |
| Root length | Mesiobuccal: 12.9 mm (8.5–18.8 mm)<br>Distobuccal: 12.2 mm (8.9–15.5 mm)<br>Palatal: 13.7 mm (10.6–17.5 mm) | Mesiobuccal: 12.9 mm (9.0–18.2 mm)<br>Distobuccal: 12.1 mm (9.0–16.3 mm)<br>Palatal: 13.5 mm (9.8–18.8 mm) |
| Number of roots | 3 (97.7%), 2 (1.8%), 4 (0.3%), 1 (0.2%) | 3 (73.7%), 2 (14.9%), 1 (10.7%), 4 (0.7%) |
| Number of canals | Mesiobuccal: 2 (60.4%), 1 (29.3%), 3 (0.1%), other (0.4%)<br>Distobuccal: 1 (98.6%), 2 (1.4%)<br>Palatal: 1 (99.26%), 2 (0.7%), other (0.04%) | Mesiobuccal: 1 (66.1%), 2 (33.7%), 3 (0.05%), other (0.2%)<br>Distobuccal: 1 (99.6%), 2 (0.4%)<br>Palatal: 1 (99.67%), 2 (0.35%), 3 (0.01%), other (0.01%) |
| Accessory canals | Mesiobuccal: 51% (coronal: 10.7%; middle: 13.1%; apical: 58.2%)<br>Distobuccal: 36% (coronal: 10.1%; middle: 12.3%; apical: 59.6%)<br>Palatal: 48% (coronal: 9.4%; middle: 11.3%; apical: 61.3%) | Mesiobuccal: 50% (coronal: 10.1%; middle: 14.1%; apical: 65.8%)<br>Distobuccal: 29% (coronal: 9.1%; middle: 13.3%; apical: 67.6%)<br>Palatal: 42% (coronal: 8.7%; middle: 11.2%; apical: 70.1%) |

Data from Versiani et al 2018a.

comparable in terms of volume and overall geometry (Versiani et al 2012). The tendency for a very wide mesiobuccal canal is not present in this tooth group (Nelson and Ash) (Table 17-6).

A great variability in root canal configuration is expected in this group of teeth, which is influenced by the morphology of the roots. Assuming the most common three-rooted anatomy, the mesiobuccal root is the most unpredictable, although the most usual root canal configuration in the first molar is the Vertucci type II, with proportions as high as 56% (Perez-Heredia et al), followed by types IV and I. For the second molar, there is an increase in prevalence for type I, which may reach more than 50%, which is followed by type II, with percentages around 30% (Perez-Heredia et al). Regarding the independent distobuccal and palatal roots, the most usual configuration is type I. Curvature to the buccal aspect is the most common finding in the palatal root (Bone and Moule); 52% of palatal roots had curvatures between 10 and 20 degrees, and 32% had curvatures greater than 20 degrees. This root can also present an absence of apical constrictions in 76% of cases (Divine et al).

Root fusions may drastically change the molar internal root canal morphology, and in such cases a reduction in the number of canals in the mesiobuccal root may be expected (Martins et al 2018a); additionally, the presence of a C-shaped configuration may be observed in both first and second molars (Martins et al 2016). Ordinola-Zapata et al (2017) evaluated the internal and external morphologies of maxillary second molars with fused roots




▶ **FIG 17-17** *(a to d)* Maxillary second molar variations. Note the three-rooted tooth with buccal fused roots in *a*. The mesiobuccal and distobuccal roots are fused in *b*, and all buccal canals including the MB2 converge into a single foramen. The distobuccal root is fused with the palatal root in *c* and *d*. Observe the presence of a C-shaped variation in *d*. (Reprinted with permission from Ordinola-Zapata et al 2017.)

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by microCT analysis (Fig 17-17). The authors found that the most prevalent root canal fusions were mesiobuccal root fused with distobuccal root (32%), distobuccal root fused with palatal root (27%), and mesiobuccal root fused with distobuccal root and palatal root fused with mesiobuccal or distobuccal root (21%). The frequency of isthmuses in the apical level varied from 9.3% to 42.8% depending on the type of root fusion, whereas the presence of apical deltas ranged from 18.5% to 57.1% of teeth (N = 100). Unlike with the mandibular molars, the authors noticed that the C-shaped configuration in maxillary second molars with fused roots rarely extended from the pulp chamber to the apex. If root-end resection is necessary, the following surgical challenges can be expected in these teeth: *(1)* Most of the variations include the fusion of the palatal root, *(2)* root-end ultrasonic preparation should include the thin isthmuses that can be present in C-shaped canals and fused roots, and *(3)* root-end resection may include simultaneous resection of three roots. In some instances of these anatomical variations, the less invasive intentional replantation technique should be considered.

**MB2 and MB3 canals.** In the last few decades, the morphology of the mesiobuccal root of maxillary molars has been more extensively studied than any other root (Weine et al). This root commonly presents two main root canals, named *MB1* and *MB2*, and a high incidence of fine anatomical structures including intercanal communications, loops, accessory canals, and apical ramifications usually result in a very complex canal system. Acosta Vigouroux and Bosaans found that the shape of the pulp chamber at floor level was found to be tetragonal in 93% of cases and that four canals were present 70% of the time. The mesiolingual (MB2) canal was often concealed by a dentinal cornice. The orifice of the MB2 is usually located either mesial to or in the subpulpal groove within 3.5 mm palatally and 2 mm mesially of MB1, often hidden under the shelf of the dentin wall or calcifications in a small groove (Martins et al 2020).

In the literature, the frequency of the MB2 canal in maxillary molars has ranged from 10% to 95%, depending not only on the method used in the study, such as sectioning, dye injection, radiography, scanning electron microscopy, or microCT, but also on ethnic and demographic factors related to the studied population, which may include geographic region, age, and gender. Kulild and Peters reported that the use of explorers and the microscope allowed the MB2 canal to be located in 96% of first molars and 93% of second molars. Furthermore, Fogel et al studied the canal configuration in the mesiobuccal root of maxillary first molars undergoing endodontic treatment by two private practice endodontists. Rhomboidal access preparations using 2.5× magnification were undertaken to ease the search for the MB2 canal. Following the subpulpal groove between the mesiobuccal and palatal orifices, a groove was created along the pulpal floor lingual to the mesiobuccal canal orifice. Troughing continued along the groove until an MB2 canal was identified or until the subpulpal groove disappeared (approximately 1 mm deep). According to the findings, 71.2% of mesiobuccal roots included two canals that might be treated. The majority of mesiobuccal roots were Vertucci types II and III at 39.4% and 31.7%, respectively. Only 28.9% were Vertucci type I.

Ordinola-Zapata et al (2019a) performed a microCT study to establish whether a relationship exists between the apical morphology of mesiobuccal roots of maxillary molars and root canal system complexity. They discovered that maxillary first molar mesiobuccal roots with aspect ratios ≥ 2 (long oval) in the axial plane 3 mm from the apex had significantly more complex root canal systems than those with aspect ratios less than 2 (more round) (Fig 17-18) (Ordinola-Zapata et al 2019a). The findings revealed that only 10% (N = 100) of the sample had a single foramen. In three cases, the MB2 foramen was coronal to the 3-mm apical level. The average distance between the apex and MB2 foramen was 1.59 mm. At least 76% of mesiobuccal roots were free of accessory anatomy at the 4-mm apical level. The authors suggested that using CBCT to determine aspect ratios ≥ 2 could be predictive of an increased number of MB2 canals, accessory canals, and apical foramina that may not always be demonstrated on the CBCT scan due to voxel size.

A recent analysis of prevalence studies using CBCT comprised data from 23,926 maxillary molars (15,285 maxillary first molars and 8,641 maxillary second molars) and reported overall MB2 frequencies of 69.6% and 39.0% in maxillary first and second molars, respectively. Additionally, a meta-analysis of data demonstrated not only that males had higher odds of having an MB2 canal than females in both maxillary molars but also significant differences among geographic regions, while age could not be associated with possible sources of heterogeneity (Martins et al 2020) (Fig 17-19).

The impact of missed canals on the outcome of endodontic treatment has already been established in the literature and can be applied to all groups of teeth. This includes a few common anatomies such as the presence of an MMC in mandibular molars, lingual canals in mandibular incisors and mandibular premolars, extra palatal canals, the MB2 canal in the maxillary molars, and a third root canal in the mesiobuccal root of maxillary molars, the so-called *MB3* (Ordinola-Zapata et al 2020) (Fig 17-20).


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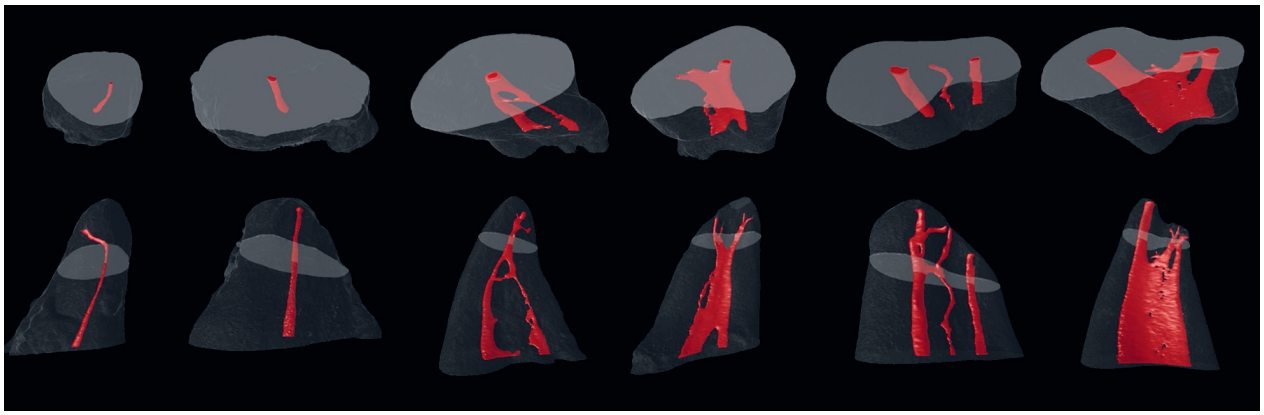
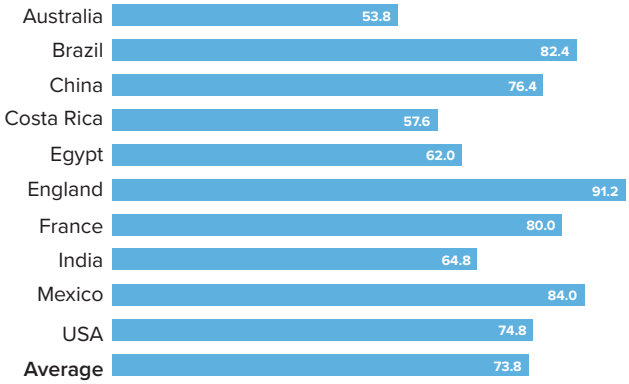
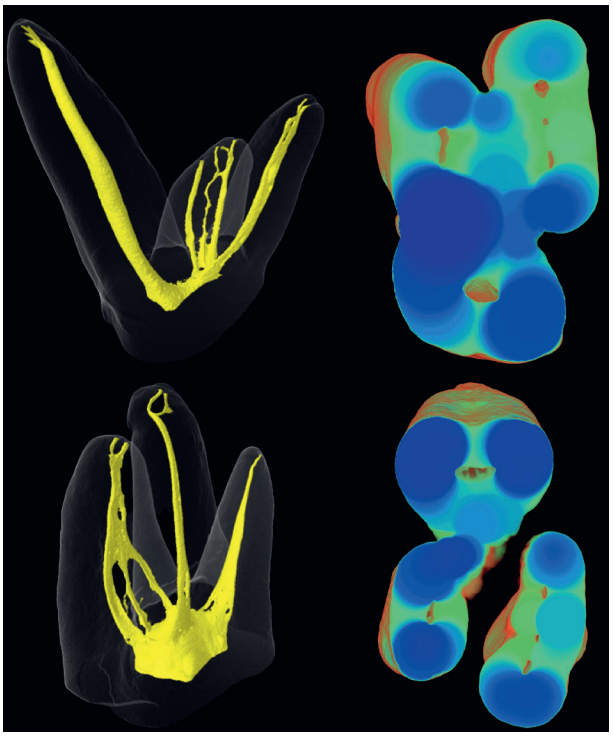


▲ **FIG 17-18** Representative microCT axial slices obtained from the apical third of mesiobuccal roots of maxillary molars. The complexity of the apical anatomy increases in long oval roots. (Modified from Ordinola-Zapata et al 2019a.)




▲ **FIG 17-19** The prevalence of MB2 canals in maxillary molars (%) in different populations. (Modified from Martins et al 2020.)



▶ **FIG 17-20** Three-rooted maxillary molars with three canals in the mesiobuccal root. (Modified from Ordinola-Zapata et al 2020.)

The *MB3 canal* can be defined as a third main root canal located between the MB1 and MB2 main canals of the mesiobuccal root of maxillary molars. Literature regarding the morphologic description of the MB3 canal is scarce, and most of the information comes in the form of clinical reports or incidental findings of laboratory studies. A recent review of the internal anatomy of maxillary molars (Tomaszewska et al) reported a global proportion of MB3 in 0.5% of 12,200 maxillary first molars and 0.3% of 4,090 maxillary second molars. However, ex vivo studies using microCT imaging systems reported a higher frequency of MB3 canals (4.9% to 10.0%) when compared with in vivo studies using CBCT (0.1% to 1.8%) or simple inspection of the pulp chamber floor in the clinic (3.1%) (Ordinola-Zapata et al 2020). In a recent study, the MB3 canal was identified in 10 of 100 three-rooted first molars (10%), 2 of 65 three-rooted second molars (3.1%), and 4 of 100 second molars with fused roots (4%) (Ordinola-Zapata et al 2020).

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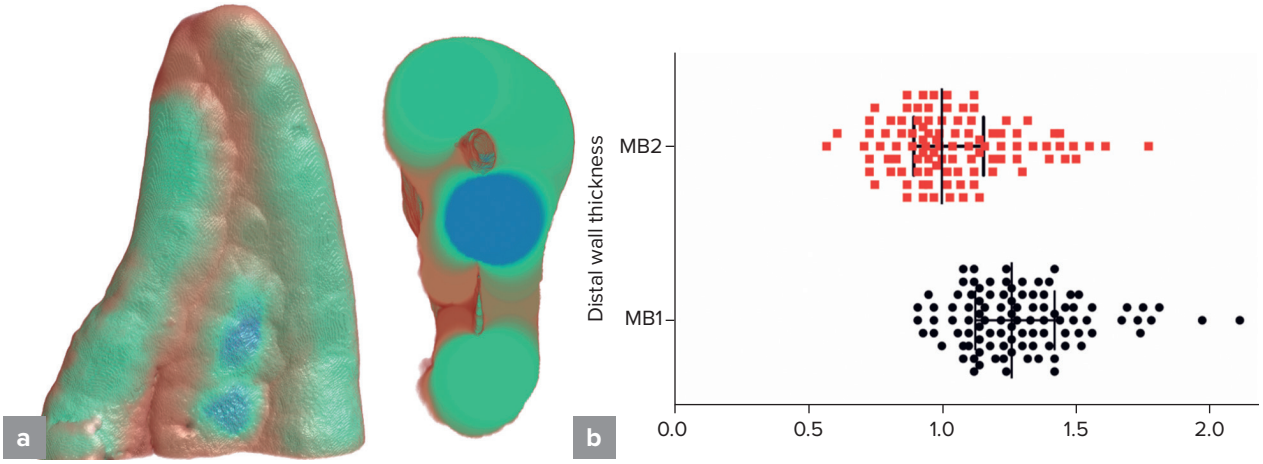


▲ **FIG 17-21** *(a)* Representative view of color-coded 3D model revealing the dentin thickness throughout the mesiobuccal root of a maxillary first molar. Thick structures are indicated in *blue* and *green*, while *red* indicates areas of thin dentin. *(b)* Dentin wall thickness measurement (in mm) of MB1 and MB2 root canals of 100 maxillary first molars at 2 mm apical to the furcation level. (Modified from Ordinola-Zapata et al 2020.)

The presence of the MB3 canal has also been related to a high variability of the root canal system configuration, including a high frequency of confluent anatomy (such as isthmuses), accessory canals, and foramina.

**Danger zone.** Although the roots and root canals of maxillary molars have been studied in detail, the danger zone in this group of teeth, which has been reported to exist in the distal portion of the mesiobuccal root, has received less attention (Degerness and Bowles, Ordinola-Zapata et al 2019b) (Fig 17-21). Harris et al used microCT technology to show that in mandibular molars, the dentin thickness on the distal part of the mesial root and 1.5 mm below the furcation region ranged from 0.81 to 1.22 mm, whereas Garala et al obtained a mean value of 1.27 mm. There was no difference in the dentin thickness measured in the mesiobuccal and mesiolingual root canals in both investigations. Ordinola-Zapata et al (2019) used microCT to examine 100 mesiobuccal roots of maxillary first molars with two mesiobuccal root canals (MB1 and MB2) and found that the dentin thickness at the distal aspect of the MB2 canal (danger zone) ranged from 0.57 to 1.77 mm and was significantly thinner than the dentin thickness of the MB1 canal on both sides of the root. Furthermore, at least half of the samples showed dentin thickness values less than 1 mm in the danger zone, which was mostly related to the MB2 canal. According to the authors, the asymmetric form of the mesiobuccal root might explain these findings.

## Demographic Aspects

Demographic aspects have been proven to be factors of heterogeneity and, consequently, relevant sources of variability in the outcome of studies on root canal anatomy. The major demographic aspects of interest are ethnicity, gender, and age, and the reasons behind their influence on dental pulp morphology vary, as explained in the next sections.

### Ethnicity

Research on the effect of ethnicity on human tooth anatomy has focused mostly on crown morphology. The number of cusps, the existence of certain cusps like the cusp of Carabelli, and other aspects of crown anatomy such as the form, fissure patterns, mesial ridges, and transverse crest have all been recorded (Bailey and Hublin). Anthropologic studies have stated that modern humans originated in Nairobi, Kenya. The northern route, through the Levant corridor bordering the Mediterranean Sea, led to the colonization of western Eurasia, while the southern route, comprising the Horn of Africa and crossing the Bab-el-Mandeb Strait, led to the colonization of India. Using different routes, humans colonized Asia, Oceania, and America. These colonization routes led to the formation of the three most relevant ethnic groups: *(1)* the Caucasians, descendants of the prehistoric individuals who settled in the Eurasian western regions; *(2)* Asians,


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descendants of the Southern route; and *(3)* Africans, who remained in the birth continent (Hanihara). Over thousands of years, these ethnic groups were exposed to multiple environmental conditions, such as nutrition, weather, and even hormonal or postnatal function differences (Hanihara, Yaacob et al), which may have led to variability selection and ultimately to distinct genome evolution and phenotypes.

In a non-exhaustive manner, Mizoguchi found that the largest-sized teeth were noted in Aboriginal Australians followed by Africans, while Asians and Caucasians tend to have smaller teeth, equivalent in size to one another. Asians have the lowest prevalence of the cusp of Carabelli in the maxillary first molar compared to Europeans and Africans (Irish, Yaacob et al). It is worth mentioning the higher prevalence of root fusions and C-shaped canal configurations in the mandibular second molar in Asians. This could reflect a possible evolutionary adaptation to the smaller jaws found in this ethnic group (Martins et al 2019b). The higher prevalence of radix entomolaris in Asians' mandibular first molars may seem counterintuitive based on the previous hypothesis; however, the presence of radix entomolaris in this population seems to be related to interbreeding and gene flow between early modern humans and extinct subspecies of archaic humans from Siberia, who were associated with mandibular molars with three-rooted configurations (Chen et al, Martins et al 2022). This evolutionary process can therefore justify differences among groups of individuals from different regions of the globe.

## Gender

Anatomical variations also appear to be strongly related with sexual dimorphism. This concept can be defined as systematic differences in the phenotypes from individuals of different gender from the same species. One source leading to sexual dimorphism is the hormonal balance, which may lead to differences in mineralized growths including bone and teeth (Alvesalo). The Y chromosome expression can lead to a higher production of enamel and dentin, while the X chromosome appears to restrict enamel formation (Alvesalo). This fact possibly explains why men's teeth have larger crowns and root length dimensions compared to women (Alvesalo, Scott et al). In addition, sexual differences may explain why a higher number of root canals are also present in males (Martins et al 2018a, Martins et al 2018b, Martins et al 2021). An important root canal system sexual dimorphism has been observed in the prevalence of mandibular second molars with C-shaped canal configurations. In certain subpopulations, the percentage of cases present in women may be up to three times higher than in men (von Zuben et al).

## Age

The explanations behind the influence of aging on root canal anatomy are different from other demographic aspects. In this case, the cause is related to the calcification process due to pathologic or physiologic reasons (Johnstone and Parashos). The deposition of secondary and tertiary dentin in response to external stimuli like caries or trauma, along with the natural aging process, can result in the formation of various calcification patterns that can affect the root canal morphology. One characteristic change is the gradual narrowing of the pulp canal space due to the continual apposition of secondary dentin (Bernick, Gani et al). Another significant mechanism occurs in long oval canals, such as those seen in the mesiobuccal root of the maxillary molar. In young individuals, early mesiodistal dentin apposition may result in the creation of the MB2 canal (Gani et al). Other patterns have been observed, and although the aging calcification process is not strong enough to radically change the root canal system configuration over the years, it can lead to the partial or complete elimination of anatomical structures though the emergence of intercanal communications (Gani et al).

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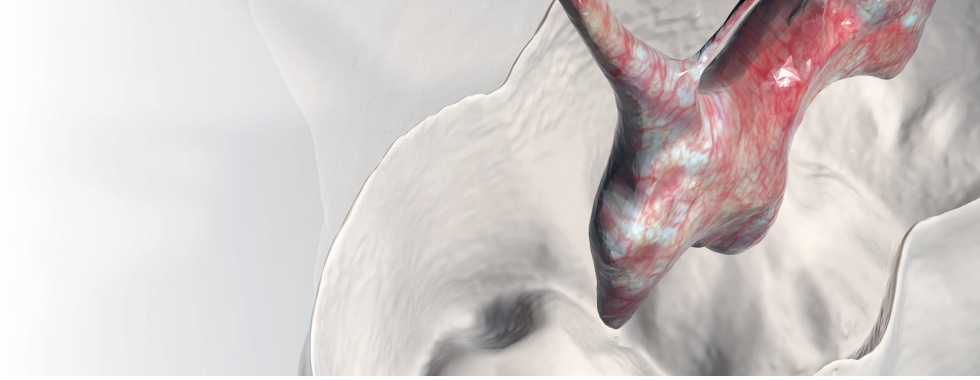

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